



Voltage Gated Sodium Channel Genes in Epilepsy: Mutations, Functional Studies, and Treatment Dimensions

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Genetic epilepsy occurs as a result of mutations in either a single gene or an interplay of different genes. These mutations have been detected in ion channel and non-ion channel genes. A noteworthy class of ion channel genes are the voltage gated sodium channels (VGSCs) that play key roles in the depolarization phase of action potentials in neurons. Of huge significance are *SCN1A*, *SCN1B*, *SCN2A*, *SCN3A*, and *SCN8A* genes that are highly expressed in the brain. Genomic studies have revealed inherited and *de novo* mutations in sodium channels that are linked to different forms of epilepsies. Due to the high frequency of sodium channel mutations in epilepsy, this review discusses the pathogenic mutations in the sodium channel genes that lead to epilepsy. In addition, it explores the functional studies on some known mutations and the clinical significance of VGSC mutations in the medical management of epilepsy. The understanding of these channel mutations may serve as a strong guide in making effective treatment decisions in patient management.

Keywords: seizures, gain-of-functions, loss-of-functions, depolarization, VGSC

INTRODUCTION

Epilepsy is a neurological disorder with spontaneous and re-occurring seizures that are borne from a population of cortical neurons, exhibiting hyper-synchronized discharge of high current action potentials in the brain (1, 2). Etiologically, epilepsy may have genetic, infectious, metabolic, structural, immunological, or unknown origin (3). Structural epilepsies are acquired following brain trauma, stroke, injuries, inflammations or infections, unlike genetic (formerly idiopathic) epilepsies which are primarily due to underlying genetic mutations (3). Studies in this field have shown that epilepsy may have a combination of two or more etiologies (4). For instance, many disorders involving the central nervous system (CNS) malformations such as tubulinopathies, tuberous sclerosis and polymicrogyria are comorbid with epilepsy and have genetic causes (5). The discovery of several ion channel gene mutations in epilepsy has led to categorizing epilepsy as a channelopathy. Epilepsy associated channel proteins fall in the class of ionotropic ligand receptors for major neurotransmitters (such as acetylcholine {muscarinic} receptors, glutamatergic receptors and GABAergic receptors) and the voltage gated ion channel proteins including sodium (Na⁺),

calcium (Ca^{2+}), potassium (K^+), and chloride (Cl^-) channels (6) are involved in bio-signaling in the CNS.

Voltage gated sodium ion channels (VGSCs) are heteromeric proteins involved in the generation and propagation of action potentials in brain neural cells (7). They are membrane-associated proteins and are responsible for conducting currents of sodium ions down their concentration gradient into the cell (8). Mutations associated with these channel genes are the most common cause of genetic epilepsies, with thousands of them reported in diverse populations (9). To understand the role of genetic variants in epilepsy, many studies have focused on the possible functional implications of a vast array of VGSC mutations. Often, these studies involved attempts to understand the mechanism by which induced mutations alter neuron physiology, using model systems. Some functional studies involved heterologous expression of mutated channel proteins in human embryonic cell lines or human induced pluripotent stem cells (hiPSCs) and the study of the aberrant network conduction in these transfected cells using voltage patch clamp techniques. Other methods involve the use of genetically modified rodent models (10) [e.g., Q54 mice, bearing the 879GAL881QQQ mutation in *SCN2A* (11)]. Several strategies for functional studies have been developed, which has helped to unravel the underlying molecular mechanisms by which many mutations trigger seizures. These have exposed other knowledge areas like the impact of mutations in modifier genes and the understanding of differences in patients' response to drugs. Therefore, this review aims to discuss VGSCs mutations in the alpha and beta subunit genes of sodium channels that lead to epilepsy, some functional studies of these mutations and their significance to epilepsy management.

SEARCH CRITERIA

A review of recent relevant literature was done on PubMed and GoogleScholar. Advanced search was done using the following keyword combinations: *SCN1A* mutations in epilepsy, *SCN1B* mutations in epilepsy, *SCN2A* mutations in epilepsy, *SCN3A* mutations in epilepsy, *SCN8A* mutations in epilepsy and functional studies of VGSCs in epilepsy. Search results (articles) containing the following word combination were excluded: Anticonvulsant properties of plant extracts, bioorganic compounds synthesized as antiepileptic drugs (AEDs), mechanism of action of new AEDs, clinical trials of drugs against epilepsy, heterocyclic compounds, imidazoles

Abbreviations: GABRG2, Gamma-aminobutyric acid receptor, gamma-2; KCNT1, Potassium channel, subfamily T, member 1; KCNQ2, Potassium channel, voltage-gated, KQT-like subfamily, member 2; FOXG1, Forkhead box G1; CDKL5, Cyclin-dependent kinase-like 5; MECP2, Methyl-CpG-binding protein 2; ARX, Aristaless-related homeobox, x-linked; *SCN1A*, Sodium channel, neuronal type I, alpha subunit; *SCN2A*, Sodium channel, voltage-gated, type II, alpha subunit; *SCN3A*, Sodium channel, voltage-gated, type III, alpha subunit; *SCN1B*, Sodium channel, voltage-gated, type I, beta subunit; *SCN8A*, Sodium channel, voltage-gated, type VIII, alpha subunit; *SCN9A*, Sodium channel, voltage-gated, type IX, alpha subunit; STXB1, Syntaxin-binding protein 1; SLC25A22, Solute carrier family 25 (mitochondrial carrier, glutamate), member 22; PCDH19, Protocadherin 19; SPTAN1, Spectrin, alpha, nonerythrocytic 1; ARHGEP9, Rho guanine nucleotide exchange factor 9.

sulfonamides with anticonvulsant properties, quantitative structure activity relationship (QSAR) models for VGSC functions, biomarker studies in epilepsy syndromes and clinical trials of AEDs.

VGSC GENES AND THEIR TISSUE DISTRIBUTIONS

There are nine different sodium channel α -subunit genes that code for Nav1.1 to Nav1.9 (12) channel proteins. *SCN1A* to *SCN5A* encode Nav1.1-Nav1.5 proteins, while *SCN8A* to *SCN11A* encode Nav1.6-Nav1.9 proteins, respectively (13). On the other hand, five different β -subunit proteins have been reported, namely: $\beta 1$ (product of *SCN1B*), $\beta 1B$ (product of *SCN1B* splice variant), $\beta 2$ (*SCN2B*), $\beta 3$ (*SCN3B*), and $\beta 4$ (*SCN4B*) (14). In humans, the expression of VGSCs follows a tissue dependent pattern and each sodium channel possesses cognate kinetic properties that are both native to the tissue and relate to its function. *SCN1A*, *SCN2A*, and *SCN3A* have their locus clustered within a 600 kb region on the long arm of the *Homo sapiens* chromosome 2q24. The following are the VGSC α subunit genes highly expressed in the human brain: *SCN1A*, *SCN2A*, *SCN3A*, and *SCN8A* (Table 1). Hence, these are the genes involved in epilepsy pathogenesis. Other sodium channels α subunit genes that have high expression in muscles are involved in other pathologies. For example, *SCN4A* mutants cause hypokalemic periodic paralysis (type 2) (15), myotonia congenita and myasthenic syndrome (16) while *SCN5A* variants result in atrial fibrillation (type 10) (17), brugada syndrome (type 1) (18), and cardiac death (19) (Table 2). As for β subunit genes, they display a wide distribution pattern in the brain. The proteins encoded by *SCN1B* (i.e., $\beta 1$ and $\beta 1B$ are developmentally regulated). The β subunits occur as transmembrane polypeptides having their amino terminus protruding extracellularly and their carboxyl terminus protruding intracellularly, interacting with the proteins of the cytoskeleton. A conserved immunoglobulin (Ig) region is located at the extracellular portion, just like cell adhesion proteins (37). These proteins are found in close interactions with cellular adhesion proteins (38). In fact, some studies have shown the adhesive property of β subunits in the brain cells (39). Apart from seizure disorders, *SCN1B* variants also cause atrial fibrillation (type 13) and brugada syndrome (type 5) (27, 30).

SCN2A, *SCN3A*, and *SCN8A* are three main α subunit genes that are mostly expressed on excitatory neurons. On inhibitory interneurons, *SCN1A* has high expression while *SCN8A* is also expressed to lesser extent (40, 41). The expression of these channels is usually concentrated on the AIS of neurons. However, *SCN2A* are mildly expressed on the soma and dendrites since they may promote back propagation of action potentials to the cell body (42). Nav1.6 is present mainly in the nodes of ranvier and distal portions of the AIS, but shows lower distribution on the dendrites and soma (43). Nav1.6 plays a higher role in action potential generation than Nav1.2 because of its relatively lower threshold for activation. Both channels differ in their functions despite the strong similarities they exhibit. Due to the

TABLE 1 | Sodium channel genes involved in epilepsy, proteins encoded, genetic loci, and distribution in human tissues.

Sodium channel gene	Protein encode	Chromosomal loci	Major tissues expressed	Major developmental stage
<i>SCN1A</i>	Nav1.1	2q24.3	Major sodium channel in inhibitory interneurons of the brain and spinal cord. Also expressed in lungs and testes.	Neonates, infant and young children
<i>SCN1B</i>	Na1.1b	19q13.11	Axon initial segment (AIS) of Inhibitory neurons of brain and heart.	Infant and young children. β 1B predominates in embryonic brain
<i>SCN2A</i>	Nav1.2	2q24.3	AIS and distal portions of principal excitatory neurons of the brain. Also expressed in kidneys.	Prenatal and infant brain, low expression on adult brain
<i>SCN3A</i>	Nav1.3	2q24.3	AIS of principal excitatory neurons of brain and adrenal tissues.	Prenatal stage, Neonates, infants, and adults
<i>SCN8A</i>	Nav1.6	12q13.13	Nodes of ranvier, AIS and distal regions of both excitatory and inhibitory neurons of the brain cortex and cerebellum.	Low in neonates, Prevalent in adults

differential expression patterns of sodium channels on inhibitory and excitatory neurons, polymorphisms in them tend to result in different seizure patterns and syndromes.

SCN1A: ROLE IN EPILEPSY AND EPILEPSY MANAGEMENT

SCN1A mutations are the most prevalent amongst all VGSCs mutations in epilepsy and over 1,250 pathogenic variants are responsible for various epilepsies (44). In fact, amongst all epilepsy genes, *SCN1A* mutations are the most implicated (6, 45). Most Dravet syndrome (DS) and Generalized Epilepsy with Febrile Seizures Plus (GEFS+) cases have mutations in *SCN1A*. Both inherited and *de novo* mutations in *SCN1A* genes cause epilepsy and epileptic encephalopathies. For example, over three-fourth of DS cases are due to *de novo* mutations in *SCN1A* genes, while *SCN1A* mutations causing GEFS+ are frequently inherited (46). Many *SCN1A* mutations cause loss-of-function [e.g., *de novo* *SCN1A* mutations leading to DS occur as loss-of-function mutations on inhibitory interneurons (6)]. These mutations decrease the activity of GABAergic inhibitory interneurons (6). *SCN1A* variants in seizure disorders may occur as various mutation types such as missense, nonsense, protein-truncating variants, etc. For instance, about 50% of all DS cases arise from missense mutation while many others arise from nonsense mutations, deletions, frameshifts and splice-site variants (47, 48). Although DS and GEFS+ can be used as prototypes to study *SCN1A* variants, these mutations are also involved in other sub-types of epilepsy (13, 49).

Initially, research done using heterologous expression systems proposed that *SCN1A* mutations cause gain-of-function arising from dysfunctional inactivation of channel (50). Later, this suggestion was challenged after a host of loss-of-function mutations were discovered in patients with GEFS+. The effect of the deleterious missense mutations often results in a change in the biophysical properties of the Nav1.1 channel, causing a positive shift in the voltage dependence of activation with or without very slow inactivation of channel (51). Using bacterial artificial chromosome (BAC) transgenic mouse model, Tang et al. (52) reported that the effect of the R1648 mutation of

SCN1A channel was a distorted resilience from inactivation, thereby implying loss-of-function. More recently, functional studies affirming loss-of-function were reported by Kluckova et al. (53) who investigated nine known and five novel variants, and observed clear partial and total loss-of-functions in different *SCN1A* variants. Four of them, E78K, E1587K, W384X, and R1596C resulted in mutant channels that failed to produce any measurable sodium currents (total function loss) while two, E788K and M909K resulted in partial loss. Three other variants (D249E, E78D, and T1934I) prevented the channel from recovering from the active state as required. Since *SCN1A* channels are majorly expressed on inhibitory interneurons, such loss-of-function mutations are expected to result in hyperexcitability.

Seizure manifestations and neuro-behavioral alterations observed in different epilepsies depend on the nature of mutations identified in sodium channels. Hence, VGSC gene screening is vital in epilepsy management. The total loss-of-function mutations in *SCN1A* results in more severe disorders. Meanwhile, the amino-acid-substituting point mutations causing change in function of sodium channels contribute to less severe epileptic disorders (6). This is exemplified in DS that arises from the loss-of-function mutation in *SCN1A* gene which is a more severe epileptic disorder than the GEFS+ (6, 9, 54). Zhang et al. (55) showed the relationship between clinical presentations of 13 children with SMEI having multiple seizure types and the incidence of having *SCN1A* mutations. Out of the 13 cases examined, 10 individuals with mutations in *SCN1A* were identified. They include 1 frameshift and 9 non-synonymous (seven missense and two non-sense) mutations. The authors concluded that despite identifying a well-known electroclinical pattern in these patients, a definitive genetic diagnostic approach involving *SCN1A* screening must be carried out for proper patient management. In another study involving 59 Italians and one Spanish patient having cryptogenic epilepsies, Zucca et al. (56) reported 12 *SCN1A* mutations. The mutational screening of *SCN1A* in 13 infants showed the presence of missense, nonsense, and frameshift mutations in patients with DS. Only 15% of the patients had abnormal brain imaging results, while most of them had normal electroencephalographic (EEG) activity (55). The consideration for genetic diagnosis in patients with

TABLE 2 | Other pathologies associated with sodium channels.

Genes	Pathological conditions	Pathology description	Inheritance pattern	References
SCN1A	Familial hemiplegic migraine-3 (FHM3)	This disorder involves severe headache triggered by sensory disturbances like flash of light, noise, etc. In FHM3, the migraine occurs with intense weakness of half of the body.	Autosomal dominant	(20)
SCN2A	Episodic ataxia, type 9	A disorder characterized by poor muscular coordination, pain, dizziness, sluggishness, poor speech, and difficulty with movement of the limbs	Autosomal dominant	(21, 22)
SCN4A	Hyperkalemic periodic paralysis, type 2	A pathologic condition involving intense bilateral weakness and loss of muscle tone as a result of elevated levels of serum potassium	Autosomal dominant	(23, 24)
	Paramyotonia congenital	Individuals with this disorder experience skeletal muscle rigidity due to inability of the myocytes to relax appropriately after contraction. It is often induced by cold or exercise and begins at an early age.	Autosomal dominant	(24)
	Congenital Myasthenic Syndrome 16	A disorder characterized with severe muscle weakness, bulbar palsy, developmental delay and respiratory issues. It arises due to aberrant signal transmission at junctions where neurons interact with muscles.	Autosomal recessive	(16, 25)
SCN5A	Hypokalemic periodic paralysis, type 2	A pathologic condition involving intense bilateral weakness as a result of reduced levels of serum potassium.	Autosomal dominant	(15, 26)
	Brugada syndrome (type 1)	This disorder causes unexpected death, often in adults. It is caused by irregular contraction of the heart ventricles	Autosomal dominant	(18, 27)
	SCN1B causes Brugada syndrome (type 5)			
	Sick sinus syndrome 1	A condition that occurs both in the elderly and in infants (congenitally). It is characterized by very slow heart beats, fainting and fatigue.	Autosomal recessive	(28)
	Long QT syndrome 3	A condition marked by arrhythmia, seizures, and/or fainting. Physiologically, it occurs when repolarisation of the heart myocytes is dysfunctional, resulting in excessively fast heart beats. A common trigger is exercise.	Autosomal dominant	(18)
	Sudden infant death syndrome	A condition characterized by spontaneous, unexplained death of a child during their 1st year of life, especially during sleep.	Autosomal recessive	(19)
	Cardiomyopathy, dilated with conduction disorder, type 1E	A progressive disorder of the heart characterized by heart enlargement, short breath, and failure to pump blood effectively.	Autosomal dominant	(29)
	Atrial fibrillation (type 10)	This disorder is more prevalent in older adults. It is marked by atrial tachycardia and arrhythmia.	Autosomal dominant	(17, 30)
SCN1B causes Atrial fibrillation (type 13)				
SCN8A	Cognitive impairment with or without cerebellar ataxia	Children with this disorder manifest intellectual delay and developmental delay.	Autosomal dominant	(31)
SCN9A	Primary Erythralgia	This condition is marked by inflammation of the extremities (hands and feet), with burning sensations, pain, swelling and redness after exposure to stress or exercise. It may begin from infancy and progress till adulthood. It is a peripheral nervous system disorder.	Autosomal dominant	(32)
	Congenital insensitivity to pain and Hereditary, Sensory and Autonomic Neuropathy(HSAN), Type IID	Persons with this condition suffer from hereditary sensory and autonomic disorder that makes them unable to sense pain and heat/cold sensations. It begins from infancy and affects other autonomic functions.	Autosomal recessive	(33, 34)
	Paroxysmal extreme pain disorder (PEPD)	PEPD is associated with painful stooling or urination and flushing of the skin. The disorder is inherited.	Autosomal dominant	(35)
	Small fiber neuropathy and neuropathic pain	Adults with this condition suffer extreme pain and or itchiness in their extremities (hands and feet), due to certain triggers like heat. It's a disorder that affects the peripheral nerves.	Autosomal dominant	(36)

epilepsy should not be teased out despite the absence of definite EEG results.

Microduplications in the 2q24.3 chromosomal region spanning through the genetic loci for *SCN1A*, *SCN2A*, and *SCN3A* that might influence expression levels of *SCN2A* were reported (57). The screened family seemed to manifest a seizure pattern that was different from the typical *KCNQ2* or *SCN2A* associated syndromes. *SCN1A* mutations showed lesser degree of electrophysiological alterations than *SCN3A* mutations. Hence, more intense phenotypes were observed in the former

(58). Also, frameshift mutations have been identified as one of the major underlying mutations in *SCN1A* responsible for DS (59). Tuncer et al. (59) reported a new *SCN1A* mutation in a 10-year-old Turkish boy who had seizure onset at 8 months old. Mutational screening of *SCN1A* revealed the presence of a deletion c.4018delC that resulted in a shift in the open reading frame, causing the premature introduction of a stop codon. The patient had a history of refractory seizures, which could not be effectively managed using monotherapy of phenobarbital, valproic acid, or other AEDs. Upon genetic screening, patient

management took the course of a combined therapy using valproic acid (VPA) and stiripentol that proved effective. The authors reported the presence of a borderline phenotype rather than the regular DS (59). Genetic investigation improves diagnosis and may help in selecting better effective combination therapies in epilepsy management.

Studies by Shi et al. (60) showed the association between single nucleotide polymorphisms (SNPs) in *SCN1A* in epileptic Chinese patients and their recovery rates when valproic acid was administered. A common *SCN1A* allele, rs3812718G>A, was linked with a positive drug response to valproic acid in the studied Chinese population (60). This was inconsistent with reports from previous studies (61) suggesting the discontinuation of sodium channel blockers as effective therapy against seizures in *SCN1A*-positive patients. This indicates that some population-based variants may alter patients' response to drugs, but this may be absent in other populations.

Fang et al. (62) reported a study of 24 epileptic patients of Chinese origin who had previously tested positive on *SCN1A* mutation screening. About 67% of the patients had missense mutations and about 17% had non-sense mutations. One patient had splice-site and frameshift mutations while large deletions were identified in only two of the patients. The patients' genetic background with respect to *SCN1A* mutations had impact on the medications that proved effective. For instance, ~33% of the patients were refractory to treatment with antiepileptic drugs (AEDs) while ~46% were drug responsive. The dietary intervention using ketogenic diet proved successful in three patients, who were rather non-responsive to AEDs. The use of sodium channel blockers proved unsuccessful in over 41% of the patients (62). Sodium channel blockers are being discontinued as a choice of treatment for the management of *SCN1A*-related seizure disorders due to their proven exacerbating effects in the presence of such mutations. In epilepsy management, the importance of mutational screening cannot be overemphasized because it gives a road map to effective diagnosis and treatment, while avoiding therapies that may aggravate the condition.

The discovery of pathogenic haploinsufficiency in DS has contributed to the clinical management of patients. Sodium channel antagonists like lamotrigine, phenytoin and carbamazepine are being discontinued in patients with DS and other epilepsy syndromes associated with *SCN1A* mutations (61). This was not the case with earlier studies on the management of *SCN1A* disorders. For instance, Krikova et al. (63) studied the effective therapeutic dose of lamotrigine (a sodium channel antagonist) in heterozygous and homozygous *SCN1A* mutations and observed the highest effective dose amongst those with homozygous *SCN1A* mutation. Today, the application of adjunctive therapies is highly sought after. However, their efficacy in the absence of adverse drug response is a matter of concern. An example is Cannabidiol (CBD) oil which has resulted in effective seizure management in patients with DS but several adverse events ranging from moderate to severe have been observed (64). New AEDs are already being considered for the treatment of DS. Two important drugs amongst these are stiripentol and fenfluramine. Although it originated in the 1970's, stiripentol is increasingly gaining attention for its minimal

adverse reactions and its capacity to effectively manage seizures in DS, epilepsy of infancy with migrating focal seizures (EIMFS) and other intractable seizures (65). The initial uproar regarding the safety of humans using fenfluramine in weight management has gradually reduced with the release of new findings showing its efficacy in seizure management for patients with DS. Ceulemans et al. (66) recorded 70% success in a study involving the treatment of patients with DS with fenfluramine. On an average, the patients stayed seizure-free for 6 years. The result of a 5-year follow-up of the same cohort, revealed the safety of fenfluramine in patients with DS with effective seizure management, and only two patients recorded cardiovascular thickening (67). Another study involving nine patients revealed that over 70% of patients on fenfluramine had reduction in motor seizures (68, 69). These studies applied fenfluramine at low doses. Lagae et al. (70) reported the outcome of a Phase III clinical trial which suggests that the drug is safe for use as a combination drug at low doses (<30 mg per day) for an extended treatment period over several years with no adverse cardiovascular effects in young patients. These results suggest a breakthrough for the treatment of *SCN1A*-seizure disorders, with DS as a prototype. More clinical trials for fenfluramine are still ongoing and interesting findings are being reported.

Using a combination of immunochemistry and immunofluorescence, Guo et al. (71) reported an increased level of expression of sodium channel alpha subunits 1 and 3, and $\beta 1$ subunits in the hippocampal region of spontaneous epileptic rats. This study revealed that cellular changes that result in the overexpression of some channel proteins may have critical roles to play in seizure generation. Also, the impact of epigenetic modifications is still gaining attention in epilepsy studies. Drugs that can specifically inhibit the activities of DNA methyltransferases (DNMTs) and Histone deacetylases (HDACs) are already being applied, especially in patients having temporal lobe epilepsy (TLE) (72). Therefore, proper understanding of epigenetic mechanisms of epileptogenesis would lead to the development of more adjunctive therapies in the management of epilepsy.

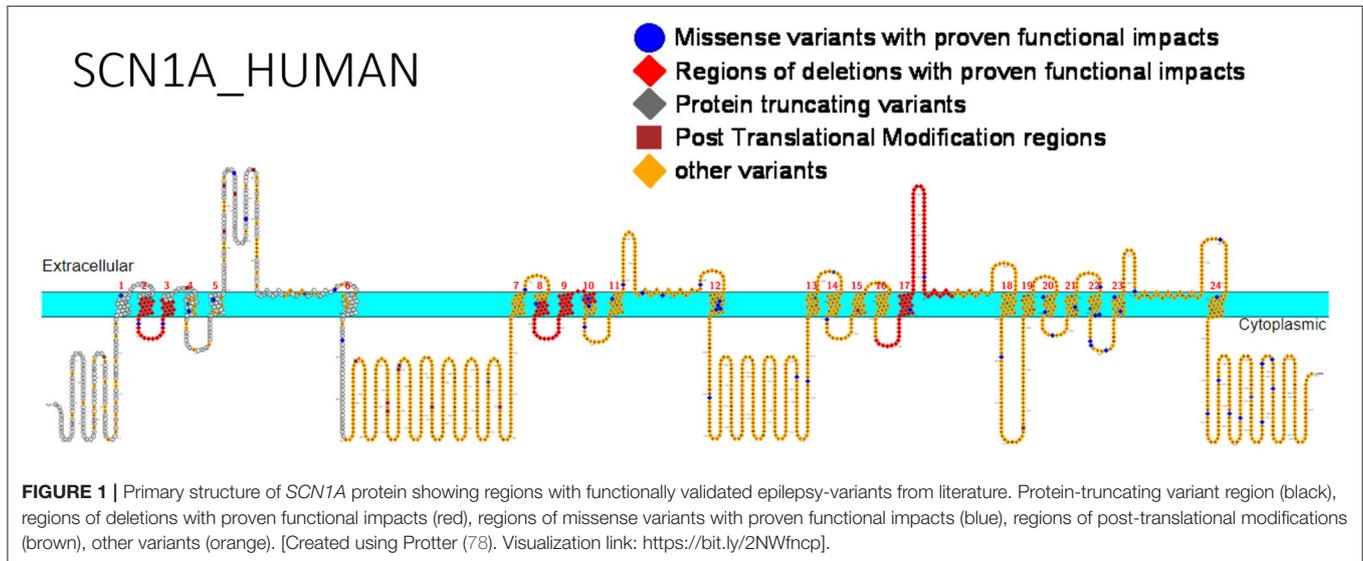
Functional studies (Table 3) can help detect gain or loss-of-function variants that may guide drug design and understanding of the relationships between variants and drug response. An example of the impact of genetic variant on drug response was recently reported by Zhao et al. (77) who examined the association between the *SCN1A* SNPs; rs3812718 and rs2298771 and the response to carbamazepine in a Chinese population. Resistance to drugs was observed with rs3812718 but not with rs2298771.

Progress is being recorded in functional studies of channel mutations. Liu et al. (48) was the first to apply neurons derived from human induced pluripotent skin fibroblasts to study functional effects of *SCN1A* mutation in a neuron-related disorder. Studies of splice-site mutation of *SCN1A* revealed clear loss-of-function using induced iPSCs. The authors compared results of using heterologous expression systems like tsA201 and xenopus oocyte cells with results of patient- and control-derived pyramidal and bipolar neurons studies. While transfected heterologous system had little or no detectable current, the

TABLE 3 | Regions of SCN1A with functionally validated epilepsy variants with references.

Gene	Amino acids affected by missense variants	Deletion regions	Protein-truncating variant regions	References
SCN1A	M145,G177,D188,I227,L263,N301,Y325, R393,Y426, E788,T808,R859,R865,T875,F902, M909,H939,R946,C959,G979,V983,N985, L986,N1011,T1174,W1204,K1270,V1353,F1415, Q1489,R1575, E1587, R1596,V1611,P1632,R1648,L1649,I1656, R1657,F1661,G1674,A1685,T1709,G1749,F1765, F1808,F1831,M1841, M1852,D1866,T1909, R1916, Q1923, R1927, T1934	V806- L863,F1289,T160- Y202,V1335- V1428,V806- L863	E78, Y159,R222,W384,R712,R1234, R1245,R1396, R1892,R1407, R1645	(44, 48, 52, 53, 73–76) and <i>SCN1A</i> database (https://scn1a.caae.org.cn/functional_studies_electrophy.php)

SCN1A database provides detailed information on functional studies of *SCN1A* mutations. Above, we have included references not yet updated on the website.



hiPSC-neurons delivered hyperexcitation as in the case with typical DS (48). They observed that hiPSCs neurons utilized the actual genetic architecture of the patients' neurons to study functional impacts rather than using other mammalian cells that may introduce some bias into the results due to inter-species differences. Also, hiPSCs permit the studying of mutations in the presence of the patient's own genetic modifiers hereby providing more effective tailor-made therapies. Furthermore, neurons are more suitable for expressing channel biophysical properties than other mammalian cells and they serve as better models. Xie et al. (44) recently demonstrated the high potential of using hiPSC-neurons for functional studies by applying the K1270 *SCN1A* mutation. They observed a reduced depolarization signal in the mutant channel which suggested loss-of-function. Likewise, they realized that this approach improved the understanding of molecular mechanism by which mutations exert their effects on neurons.

Genetic screening may reveal population-based variants responsible for differences in response to drugs in diverse populations. One advantage of identifying population-related variants with their functional studies is that it will pave way for

the development or determination of drugs that are more suitable for that population.

We have collated the *SCN1A* regions having variants whose functional studies have been reported in **Figure 1**, while **Table 3** shows the affected regions with references.

SCN1B: ROLE IN EPILEPSY AND EPILEPSY MANAGEMENT

Mutations in the modulating β subunits of the type 1 sodium channel genes have equally been confirmed as another cause of epilepsy. Wallace et al. (79) was the first to report a pathogenic missense mutation, p.(C121W), in *SCN1B* gene inherited in an autosomal dominant pattern in an Australian GEFs+ patient. Studies using heterologous expression systems revealed that this mutation hindered the function of β subunits both as an ancillary protein and adhesive protein in addition to altering gating characteristics of the channel. It also disrupts the Ig loop of the subunit, hereby inhibiting glycosylation (38, 80–82). Many mutations have been identified in this gene since then.

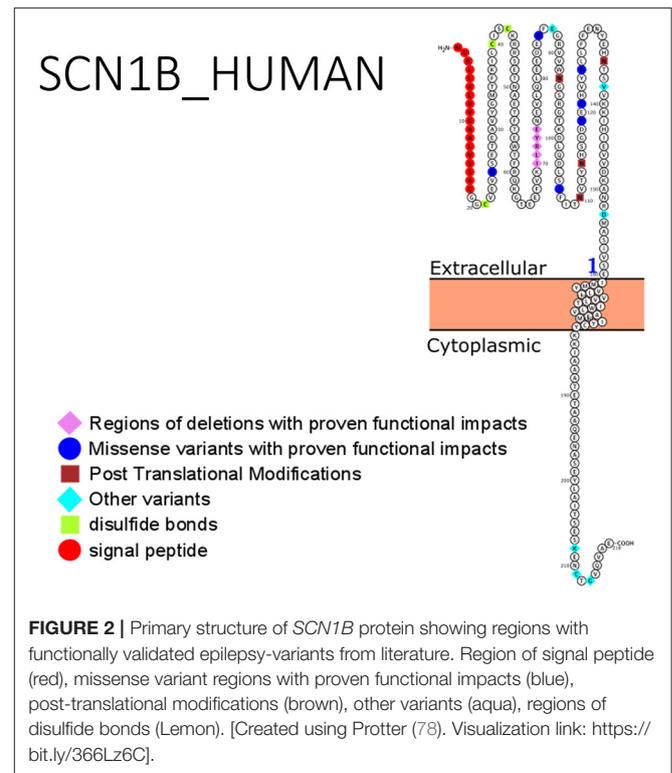
One homozygous mutation was reported in a case of epileptic encephalopathy (83). Another study identified four mutations in *SCN1B* having linkage to epileptic seizures. All these mutations were found on the extracellular portion of the protein (84). This portion on the modulating β -subunit was essential in channel gating. The four mutations were identified in patients having GEFS+. The most occurring missense substitution was the point mutation replacing cysteine with tryptophan p.(C121W) (84). More insights into the roles of sodium channel β subunits have shown that they do more than simply modify the functions of the α -subunits. In the absence of α subunits, they can function as indicator molecules in electrically activated cells. In addition, they play key roles in brain morphogenesis, modification of potassium channels, regulation of channel expression and cell to cell interactions (10).

Mutations in *SCN1B* have been reported in patients having GEFS+ phenotype, DS and Temporal lobe epilepsy (TLE) (79, 84, 85). However, many of these mutations are localized in the extracellular region of the auxiliary channel protein. Most functional studies on *SCN1B* revealed loss-of-function mutations, especially interfering with their ability to augment VGSC gating (79). The functional implication of these β subunit mutations is that they may impede the role of the principal pore forming α -subunits (86). Meanwhile, molecular studies to unravel definite pathogenicity of mutations in *SCN1B* are still incomplete.

Kruger et al. (87) reported the functional studies of the missense mutation, p.(C121W) that was initially reported by Wallace et al. (79). Using an experimental model of childhood febrile seizures, the authors compared the response of heterozygous *SCN1B*-C121W (*SCN1B*+/*w*) mice with *SCN1B*-null allele. The *SCN1B*+/*w* mice exhibited higher predisposition to heat-induced paroxysms, suggesting a gain-of-function mechanism (87). The results were contrary to the initial conclusions from co-expression studies using *Xenopus* oocytes and mammalian cell lines that suggested a loss-of-function of channel protein as a mechanism of action for this mutation (38, 79, 80). The response of the channels to the sodium ion channel blocker; phenytoin, decreased in the presence of the *SCN1B* mutation p.(C121W) (88).

Patino et al. (89) examined the functional implications of the mutation, p.G257R, earlier identified by Patino et al. (83) in β 1B subunit which is mainly expressed in embryonic brains. They demonstrated that unlike β 1, the splice-variant, β 1B, does not function as a transmembrane polypeptide. Rather, it served as a secreted protein acting as a ligand in neuronal growth. Furthermore, they revealed that the mutation might alter sodium ion currents in the brain, using Chinese Hamster Ovary cells co-expressing Nav1.3 and β 1B subunits. Similarly, their results suggested that p.(G257R) mutation acted in a similar manner to a previously reported missense mutation p.(R125C) which inhibited the release of β 1B polypeptide into the extracellular environment, hence annulling its function in neuronite outgrowth (89).

Studies involving the mutant *SCN1B* gene in heterologous expression systems require co-expression with the α -subunit gene. Most mutations in *SCN1B* are found on the extracellular



immunoglobulin fold (whose primary role is in cell to cell adhesion and interaction with the α -subunits) with few exceptions (e.g., one mutation on β 1B subunit was identified in its C-terminal domain). Phenotypic expression of these mutations and functional characterization suggested a loss-of-function mechanism. Additionally, the β 1 subunit had interactions with some types of potassium channels, thus aiding repolarization of these channels (90), by accelerating the activation of the potassium channel and suppressing its inactivation. In GEFS+ patients, where variants p.(C121W) and p.(R85C) of *SCN1B* have been described, there was a loss of effects of β 1 function on the Kv1.3 potassium channel (91). Identification and characterization of the roles of β 1 and β 1B variants in other forms of epilepsy, especially their effects on channel gating are of crucial importance. The role of *SCN1B* was recently demonstrated in developmental and epileptic encephalopathy. The mutation, R85C of *SCN1B* was identified as a loss-of-function variant (92). This was verified by Dang et al. (93) who studied the missense mutations: p.(R85C), p.(R85H), and p.(R89H). Dang et al. (93) confirmed a loss-of-function mechanism for the R85 variants. Clinical management of a child with refractory seizures proved ineffective with several combination therapies including levetiracetam, clobazam, Valproate and topiramate. However, the administration of Phenytoin resulted in effective management of the seizures (93). Despite this finding, the treatment of *SCN1B*-related epilepsy with sodium channel blockers remains inconclusive. There is still sparse information on the successful treatment in *SCN1B*-related epilepsy. Hence, more work is required, including functional studies using induced hIPSCs.

TABLE 4 | Regions of *SCN1B* with functionally validated epilepsy variants with references.

Gene	Amino acids affected by missense variants	Deletion regions	Protein-truncating variant regions	References
<i>SCN1B</i>	D25, R85, R89, I106, Y119, R125, C121	I70-E74	-	(79, 83, 85, 87, 94–97)

Figure 2 shows *SCN1B* primary structure with regions having seizure-prone variants that have been functionally validated from literature while **Table 4** shows affected regions with references.

SCN2A: ROLE IN EPILEPSY AND EPILEPSY MANAGEMENT

There are two isoforms of *SCN2A*: one is expressed in infancy while the other is expressed in adulthood. The adult isoform has lower threshold for excitation than those of a neonate, thus, it is more excitable (10). Reid et al. (98) reported about 20 pathogenic variants of *SCN2A* leading to benign familial neonatal infantile seizure (BFNIS) and GEFS+. The pathogenic mutations in this gene have been reported in several epilepsies of different severities. There is an age dependent seizure resolution in BFNIS that relates to the physiological reorganization of the axon initial segment during development. This rearrangement leads to the substitution of *SCN2A* with *SCN8A* in the distal regions of the AIS. Thus, reducing the impact of the mutant *SCN2A* on neuronal function (99). *SCN2A* and *SCN8A* mutations have been reported in some patients with severe epileptic encephalopathies (100). Ogiwara et al. (101) reported another more severe epileptic encephalopathy arising from *de novo* *SCN2A* mutations with clinical manifestations that resemble those of DS. Whole exome sequencing is continuously having more relevance in epilepsy genetics, especially in its potential to reveal genes whose mutations may have epistatic interactions by acting as genetic modifiers of disease. Studies by Shi et al. (102) to examine the impacts of *SCN2A*, *GABRG2*, and *SCN1A* in DS amongst Japanese population showed 29 mutations in *SCN1A* and 3 in *SCN2A*. The missense mutations, F328V and D322N were inherited while R1312T was *de novo* (102). Many *SCN2A* mutations leading to refractory epilepsies are often *de novo* rather than inherited (103).

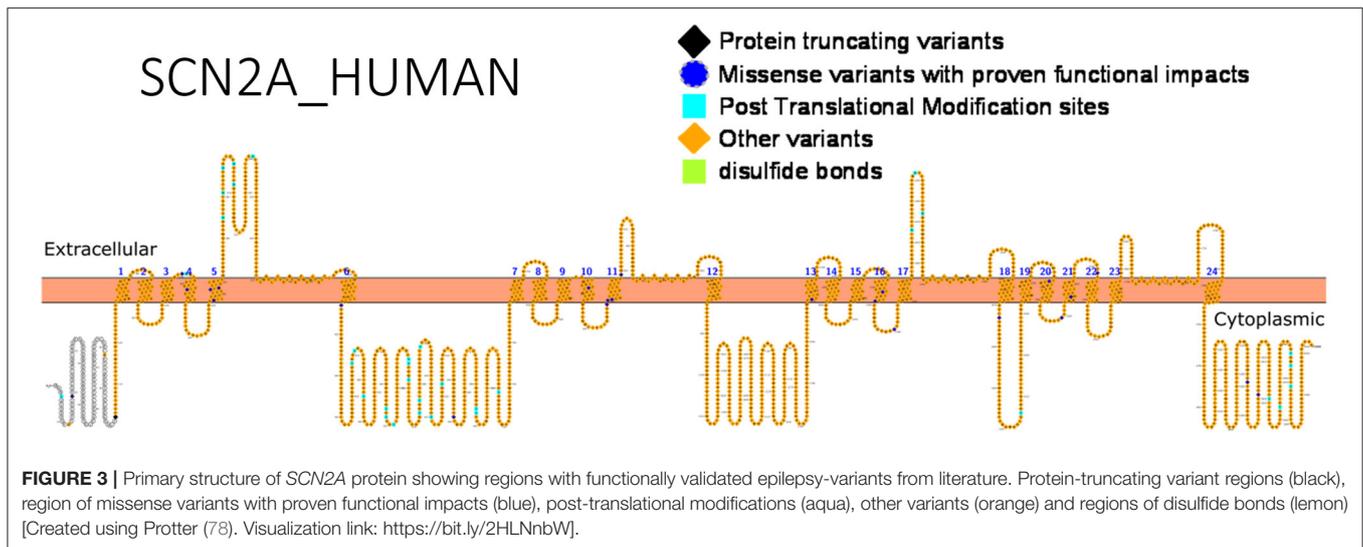
Frameshift mutations have been noted in *SCN2A* in children having intellectual disability (ID) without seizure history (104). The first non-sense, protein-truncating mutation p.(R102X) in *SCN2A* was reported in patients having refractory seizure and ID by Kamiya et al. (105). This mutation was heterozygous and absent in parents of the reported patients. Electrophysiologic analysis using HEK 293 cells and voltage patch clamp studies showed that the mutation caused a hyperpolarization shift. Liao et al. (99) examined the clinical presentations in one child with neonatal seizures accompanied by pain and episodic ataxia. The authors reported a missense mutation p.(A263V) whose functional consequence is a rise in sodium currents in the mutant channel (99). Despite these promising results, mutation studies of

SCN2A in patients with seizure disorders have not shown clearly defined phenotypes unlike *SCN1A* and *SCN8A* (106).

The role of *SCN2A* mutations in Early Onset Epileptic Encephalopathy (EOEE) was reported by Nakamura et al. (107) in a study involving 328 individuals with various types of EE. Fifteen novel amino acid replacing mutations were reported with ~86% (12) occurring *de novo*. Amongst the cases studied, all mutations in patients having Ohtahara syndrome occurred in the polypeptide loop that connects two transmembrane regions of Nav1.2. A country-wide study of patients with acute encephalopathy and fever-induced status epilepticus was recorded. Two amino acid substituting mutations in *SCN2A* were identified, one was previously identified in patients with DS p.(F328V) and the other was novel p.(I172V) (108).

Missense mutations in *SCN2A* have shown varying effects on the mutant protein. Cell culture studies using two BFNIS gene mutations revealed a depolarizing shift in voltage dependence of inactivation and an elevated/persistent current generation depicting gain-of-function as one mechanism in which *SCN2A* mutations cause excitability (109). Gain-of-function mutations in Nav1.2 cause seizures since these sodium channels are highly expressed on excitatory neurons. In a study conducted by Xu, Thomas (110) it was evident that gain-of-function mutations of the neonatal Nav1.2 predisposes to a higher seizure frequency than those of adult. The mutation in neonatal Nav1.2 increased excitability to a level that was similar to that expressed by the adult phenotype (110). This explains the self-limiting effect of BFNIS with advancement in age (10). Further studies are required to identify and characterize more *SCN2A* mutations and their involvements in epilepsies.

SCN2A mutations have been shown to be the second most prevalent mutation following *KCNT1* (111) in patients having EIMFS. Treatment with phenytoin at high dosage proved effective in 75% of the cases having multiple epileptic syndromes including EIMFS and OS (112). The authors reported 11 missense mutations in *SCN2A* including a deletion mutation, that resulted in an amino acid substitution (112). Reports from Matalon et al. (113) supported the claim that *SCN2A* mutations are involved in early infantile epilepsies. They presented three children having refractory seizures with *de novo* *SCN2A* mutations. The three patients reported were found to be negative for mutational screening in the following epilepsy genes: *FOXG1*, *CDKL5*, *MECP2*, *ARX*, *SCN1A*, *STXBPI*, *SLC25A22*, *PCDH19*, *SPTAN1*, and *ARHGEF9*. The study showed that *SCN2A* does not merely act as a disease modifying gene but can independently trigger seizures. Three amino acid substituting *de novo* mutations were reported (113). Exome studies by Baasch et al. (103) revealed one new *de novo* missense mutation p.(R1882L) in *SCN2A* in patients with epilepsy and intellectual disability. The authors presented



another supporting claim that pathogenic *SCN2A* mutations were often *de novo*.

Zeng et al. (114) reported the clinical phenotypes of 21 children having mutations in *SCN2A*. Approximately 48% of the cases had inherited the mutations from their parents while the remaining had *de novo* mutations in *SCN2A*. Majority (about 86%) of the children had partial seizures and most of the patients (about 90%) manifested more than one seizure type. Less than one-third of the examined patients had infantile spasms. *De novo* mutations in this gene were found to be associated with delayed development.

Seizures arising from *SCN2A* mutations are well-controlled by high therapeutic doses of the sodium channel blocker, phenytoin (112). Two other sodium channel antagonistic seizure medications (lidocaine and mexiletine) were reported to be effective anecdotally in infants having intractable epilepsy due to *de novo* mutations in *SCN2A* (115). This was after a combination therapy including topiramate, levetiracetam, lacosamide, oxcarbazepine, and phenytoin proved abortive. Ketogenic diet including adjunctive therapies involving treatments with pyridoxine, folic acid, pyridoxyl-5-phosphate was also applied to no avail. This medication was replicated in another patient carrying a *de novo* *SCN2A* missense mutation p.(G999L) and a successful seizure management was recorded (115). Conflicting reports in the management of *SCN2A*-related seizures were recorded until 2017 when Wolff et al. (116) identified a trend in the functional studies of *SCN2A*-variants. They reported that loss-of-function in *SCN2A* variants were more associated with seizures beginning over 3 months of age and were not properly managed using sodium channel antagonists. However, in infants <3 months a gain-of-function trend was observed and the administration of sodium channel blockers proved very effective (116). This trend requires further studies for clear validation and clinical applications.

As in the case with *SCN1A* mutations, dietary interventions are sometimes effective in managing *SCN2A*-related seizures. The use of modified Atkins diet in one Chinese child was found to

reduce seizures that presented with refractory seizures, infantile spasms and delayed development including autism-related characteristics. An *SCN2A* mutation p.(E1211K) which had been earlier identified in an infantile spasm case was detected (117). In spite of past failed attempts in the use of ketogenic diets in managing *SCN2A*-seizures (115), ketogenic diet has proved successful in managing *SCN2A*-epilepsy when adopted early enough (118, 119).

Apart from determining the treatment route, genetic variants may also determine drug response, and this has been reported for *SCN2A*. An association between *SCN2A* variants and selective drug response to valproic acid (VPA) was recently reported by Shi et al. (60) in Chinese patients. The allele, rs2304016A>G was negatively associated with response to VPA. Epistatic interactions amongst genes may equally determine how patients respond to drugs. The impact of epistatic interactions affecting response to valproic acid was studied in Chinese Han population (120). The following *SCN2A* SNPs had epistatic interactions influencing VPA response: rs10197716 and rs2119068, rs10197716, and rs11889342. Furthermore, interactions between *SCN2A* (rs7598931) and UDP-Glucuronosyltransferase-2B7 gene (*UGT2B7*) (rs12233719) were reported (120). Functional studies can help detect gain- or loss-of-function variants that can guide drug design, identify high risk population-related variants that are important for genetic testing and understand relationships between variants and drug response. **Figure 3** shows *SCN2A* primary structure with regions having variants have been functionally validated from literature while **Table 5** shows the affected regions with references.

SCN3A: ROLE IN EPILEPSY AND EPILEPSY MANAGEMENT

The *SCN3A* gene is highly expressed in brains of neonates and adults. It is similarly expressed both in principal excitatory and inhibitory interneurons. Point mutations in *SCN3A* genes

TABLE 5 | Regions of *SCN2A* with functionally validated epilepsy variants with references.

Gene	Amino acids affected by missense variants	Deletion regions	Protein-truncating variant regions	References
<i>SCN2A</i>	R19, R1902, R1882, R853, L1563, R1312, Y1589, R1319, L1563, L1330, M252, V261, A263, I1473, E1211, D649, F1597, V423, G899, N1622, G879-L881, G211, R223	-	R102	(11, 99, 101, 109, 121, 122)

have been reported in patients with focal epilepsies (58, 123). Holland et al. (12) presented the first *SCN3A* mutations in cryptogenic focal seizures. In managing the patient's seizures, previous treatments that involved the administration of sodium channel blockers (carbamazepine or oxcarbazepine) had been futile. After mutational screening, the authors reported 1 new missense mutation (K354Q) in the polypeptide link between S5-S6 of domain I, and 3 novel synonymous variants in *SCN3A*. Electrophysiological studies of the missense mutation using *SCN5A* gene show a rise in sodium ion currents causing defects in inactivation in excitatory neurons. Although this suggests that the mutation acts through a gain-of function mechanism, the use of an isoform rather than *SCN3A* itself, may introduce a bias in the accurate measurement of the effect of this mutation. The first mechanistic study of *SCN3A* mutation using transfected neurons of the hippocampus was reported in 2010. Estacion et al. (124) provided the functional studies of the p.(K354Q) mutation (earlier reported by Holland et al. in 2008) in Nav1.3 channels using hippocampal cells from rats. The authors validated that this mutation increased ionic currents across the channel and decreased the threshold for Na⁺, resulting in hyperexcitation. A more improved approach now involves the use of neurons derived from patients' fibroblasts to express mutant channels and better results are being recorded in terms of studying the channels' biophysical properties. Functional studies of even more *SCN3A* mutations have shown an elevated ramp current in mutant channels, further suggesting gain-of-function (123).

Apart from gain-of-functions mutations, loss-of-functions mutations are implicated in focal epilepsies. Chen et al. (58) reported an *SCN3A* mutation in some patients with GEFS+. The identified mutation was consequential in creating a depolarizing shift in voltage-dependent activation and inactivation of the sodium channel, including delayed recovery from slow inactivation. Hence resulting in channel inactivity. Furthermore, Lamar et al. (125) identified a novel mutation in *SCN3A* yielding the mutant Nav1.3 protein p.(L247P) which is associated with focal epilepsy in childhood and delayed development. The authors demonstrated the role of a novel *SCN3A* mutation p.(L247P) in seizure pathogenesis. They reported that loss-of-function mutation in *SCN3A*, reduced trafficking of channel proteins or down regulated expression may result in epilepsy. Despite this, more studies have shown a prevalence of gain-of-function mutations for *SCN3A*. Zaman et al. (126) studied some *SCN3A* missense variants including p.(I875T), p.(P1333L), p.(V1769A), p.(R1642C), and p.(L1799Q). The result of biophysical studies on channel mutation showed a prevalence

of gain-of-function with higher amplitude of inactivation and a lowered threshold of activating the channel compared to the wildtype channel. This effect was impeded by the application of 2 specific sodium channel blockers (lacosamide and phenytoin). Thus, implying their efficacy in managing *SCN3A* disorders. Mixed results of gain and loss-of-function are recorded because *SCN3A* is known to be expressed also on inhibitory neurons and this may explain why loss-of-function in this gene may also result in seizures.

Microduplication mutations have likewise been implicated as epilepsy-related *SCN3A* mutations. Yoshitomi et al. (127) reported 3 patients manifesting focal seizures and infantile spasms having microduplication mutation on the 2q24.3 chromosomal loci. Eight additional duplications were reported by Thuresson et al. (128). The region duplicated was overlapped and the spanned range approximately between 0.05 and 7.63 Mb in size. This resulted in an extra *SCN2A* copy which caused benign familial infantile seizures (BFIS), but had no link with cognitive decline (128). Recently, the role of deletion mutations has been expounded. A causative *de novo* deletion mutations in the 2q24.3 region was reported in a patient with West syndrome who also manifested symptoms of autism. This deletion (~1.1 Mb) covers the region encoding Nav1.2 and Nav1.3 (129).

Apart from *de novo* mutations, inherited mutations are known to cause *SCN3A*-seizure disorders. Using targeted exome sequencing including 412 epilepsy genes in 63 patients and their families, an inherited missense mutation p.(R621C) was identified in *SCN3A* but no functional studies was done (130). Zaman et al. (126) asserted that since many reported *SCN3A* variants occur *de novo*, they might have roles in modifying the effects of other pathogenic variants from different genes, which exhibit high penetrance. Functional analysis of these channel variants will assist in unraveling the molecular mechanism underlying phenotypic expression and shed more light on research to develop more effective therapies. **Figure 4** shows *SCN3A* primary structure with regions having variants that have been functionally validated from literature while **Table 6** shows the affected regions with references.

SCN8A: ROLE IN EPILEPSY AND EPILEPSY MANAGEMENT

Mutations in *SCN8A* gene is the major underlying factor responsible for pathogenesis of *SCN8A* encephalopathies;

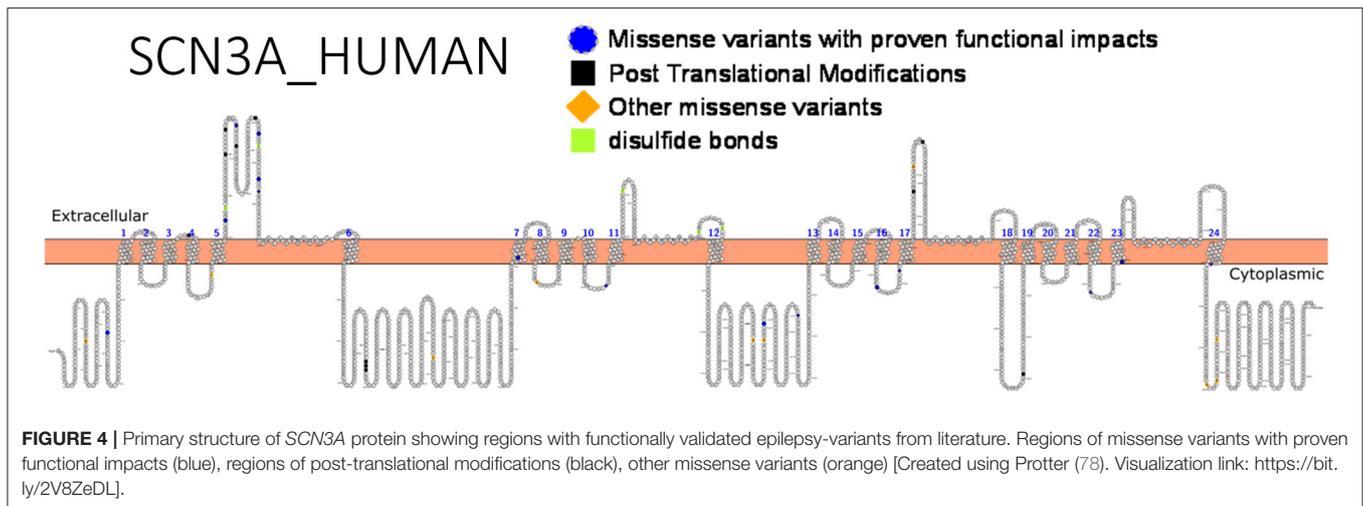


TABLE 6 | Regions of *SCN3A* with functionally validated epilepsy variants with references.

Gene	Amino acids affected by missense variants	Deletion regions	Protein-truncating variant regions	References
<i>SCN3A</i>	I85, N302, R274, A343, K354, R357, I766, I875, D1111, V1323, P1333, R1642, N1657, V1769, E1160	-	-	(12, 124–126)

a seizure disorder marked with early onset paroxysmal manifestations characterized by hypersynchronous electrical discharges originating in the brain. This usually occurs within the first 18 months after birth, particularly after the 3rd month (131). Clinical manifestations include cognitive disability and developmental delay. About half of the children involved did not walk or sit properly (or not at all), and often manifest defects with the peripheral limbic functions. One-tenth of them have experienced sudden unexpected death in epilepsy (SUDEP). The antiepileptic drugs in the class of sodium channel blockers often guarantee transient improvement in few of the affected patients. However, the control of the paroxysmal discharge is far from sufficient (132).

Recent works in genomics have identified *SCN8A* as an epilepsy gene. Nav1.6 are widely distributed on the AIS of principal excitatory neurons and inhibitory interneurons (133). Expression of Nav1.6 is not limited to the CNS but is largely expressed in PNS neural cells. The expression of *SCN8A* is highly concentrated at the AIS of both excitatory and inhibitory neurons (48). In both neuron types, Nav1.6 coordinates excitation of neurons (43, 134). Studies using *SCN8A* knockout mice revealed that this gene is primarily involved in neuronal excitability in excitable cells such as purkinje cells of the cerebellum, ganglion cells of the retina and hippocampal cells (135). Hence, these mice exhibit early (juvenile) death (136). Furthermore, Nav1.6 is the main VGSC at nodes of Ranvier of mature neurons (43), and its under-expression inhibited nerve conduction (137).

Initial genetic studies using rodents revealed that the *SCN8A* gene is also mutated in epilepsy with absence seizures, a genetic generalized epilepsy (GGE) (138). Papale et al.

(138) was the first to demonstrate that *SCN8A* is also a candidate gene in epilepsy using a mouse model with absence epilepsy. Heterozygous *SCN8A*-knock out mice manifested unprovoked spike-wave discharges which is typical of absence epileptic seizures.

Early infantile epileptic encephalopathy Type 13 (also called *SCN8A* encephalopathy) is caused by mutations of the *SCN8A* gene encoding the alpha subunit of Nav1.6 channel protein (132). Family studies have repeatedly shown *de novo* mutation in affected individuals, often arising from gain-of-function missense non-synonymous mutations. The mutant sodium channel exhibit enhanced activity (139). In a report by Blanchard et al., the physiological change observed in a mutant Nav1.6 was dysfunctional channel inactivation and a hyperpolarizing shift in voltage dependence of excitation resulting in an untimely or early channel re-opening (140). This implies that such mutations are localized on the Nav1.6 channels located on the principal excitatory neurons.

Veeramah et al. (141) reported a case of *de novo* heterozygous missense mutation in a child with severe infantile encephalopathy which started at 6 months. It was characterized with epileptic convulsions during the 4th year and was associated with SUDEP at age 15. This was a p.(N1768D) mutation in the Nav1.6 sodium channel expressed in the brain. Upon the expression of this mutant gene in ND7 cells, an enhanced ramp current with a corresponding shift in quick inactivation that was in favor of depolarization was observed. This led to hyperexcitability in pyramidal neurons of the hippocampus. In addition to this, incomplete inactivation of the channel was detected and there was a depolarizing shift in the voltage dependence of inactivation of the mutant channel (141).

Complete identification and characterization of this mutation was typical of a gain-of-function, associated with enhanced ramp current. Hence, they suggested that a gain-of-function mutation in *SCN8A* can cause epileptic encephalopathy. The implication of this new mutation was a dysfunctional channel with an impaired inactivation potential leading to neuronal hyperactivity. Several large exome sequencing projects have equally identified 11 *de novo* mutations of *SCN8A* in singleton patients with epilepsy with or without intellectual disability (135, 142). In addition, another *de novo* mutation of *SCN8A* was discovered in a child having epileptic encephalopathy with congenital abnormalities (143).

Prior to this, a loss-of-function frameshift mutation (also identified in parents) caused by a 2 bp deletion p.(Pro1719ArgfsX6) was detected as a possible underlying factor for cerebellar ataxia associated with cognitive problems (144). Another study also identified seven potentially pathogenic variants in *SCN8A*, in patients manifesting seizures and intellectual disorder (142). Four of them were *de novo* and uninherited from any of their parents, another was paternally inherited (father was asymptomatic), while the last two were unknown. Studies on *SCN8A* null mice revealed reduced excitation in the hippocampal neurons (145) while experimental works to overexpress *SCN8A* in excitatory neurons were associated with seizures (146).

The understanding of the role of *SCN8A* in seizure initiation and epilepsy became well-known following the detection of *de novo* mutations in patients having epileptic encephalopathies with varying seizure severities. Another heterozygous mutation c.2300C>T in *SCN8A* was identified in a patient possessing delayed development, cognitive disability, and unmanageable seizures. This produced a mutated Nav1.6 polypeptide with a substituted amino acid p.(T767I) (100). Functional characterization of this mutation further revealed a gain-of-function, with a hyperpolarizing shift (10 mV) in voltage dependence of activation, including a rise in ramp current. These works enhanced the understanding of the gain-of-function roles of *SCN8A* mutations in epileptic encephalopathies, and more importantly, that *SCN8A* is a causative gene in children epilepsies. The works of Pan and Cummins (147) corroborate the gain-of-function theory, thereby suggesting a hyperpolarization-shift as a mechanism of action for the R850Q mutation. They further applied and recommended the use of computational simulation to predict the impact of variants on channel function.

However, loss-of-function mutations had been experimentally implicated in *SCN8A*. de Kovel et al. (148) demonstrated that the loss-of-function missense mutation in Nav1.6 p.(R223G) on inhibitory interneurons is another cause of infantile epileptic encephalopathies. Functional characterization studies showed a loss-of-function depicted by a significant decrease in the amplitude of current. Both gain-of-function mutations affecting Nav1.6 channels on principal excitatory neurons and loss-of-function mutations that affect Nav1.6 channels on inhibitory interneurons may lead to seizure generation.

Sprissler et al. (149) identified a new drug target and expanded the pathogenesis of *SCN8A* encephalopathies by comparing gene

expression patterns before and after seizure onset and afterwards in a mouse model of early infantile epileptic encephalopathy Type 13 (149). The mutation, p.(N1768D) was introduced into the genome of the mouse by TALEN targeting. These knock-in mice exactly demonstrated the seizure disorder around the 3rd and 4th months of life (139). In addition, developmental disabilities were expressed in the mice, characterized by skewed postures, impeded movements, and tremors, which are typical of *SCN8A* encephalopathies. This further establish that either gain or loss-of-function mutations in *SCN8A* cause epilepsy. Recent studies show that loss-of-function mutations predispose to cognitive decline and intellectual disability without seizures while gain-of-function mutations result in more severe phenotypes associated with intractable seizures (150). More functional studies (using neurons) that will corroborate the findings of Liu et al. (150) are required to establish these genotype-phenotype relationships.

The pharmacological response of patients with gain-of-function mutations in *SCN8A* has been studied (151). Using a study involving four patients with *SCN8A* mutations, the authors discovered that sodium channel antagonists such as phenytoin at high therapeutic doses effectively manage *SCN8A* seizures. This is true in the case of a Chinese girl bearing the *SCN8A* mutation, p.(N1318S), in whom the administration of sodium channel antagonists proved successful (152).

A difference in drug response can occur in patients with different sodium channel mutations as observed in the cases treatment of *SCN1A* and *SCN8A* related seizures with sodium channel blockers. The former resulted in exacerbated seizures while the latter led to seizure mitigation. Hence, genetic testing is indispensable. Functional studies can help detect gain or loss-of-function variants that can guide drug design, identify high risk, population-related variants that are important for genetic testing and understand relationships between variants and specific drug response. Functional validation has been done for almost 20 different *SCN8A* variants (147). **Figure 5** shows *SCN8A* primary structure with regions having variants have been functionally validated from literature while **Table 7** shows the affected regions with references.

SCN9A: AN EPILEPSY GENE OR NOT?

The sodium channel; Nav1.7 is expressed in the brain stem, cerebral cortex and spinal cord, but its major expression is in the peripheral nervous system (PNS) (155). Some *SCN9A* variants have been found in epilepsy and autism spectrum disorders. Also, many mutations in this gene are related to inherited chronic pain syndromes, erythralgia, familial rectal pain (156, 157). Some *SCN9A* missense mutations have also been reported in patients with DS. Because the first *SCN9A* mutations identified occurred concurrently with *SCN1A* mutations, they were initially categorized as “genes with modifying roles to *SCN1A*” on inhibitory interneurons. But later on, six missense mutations were discovered in patients with febrile seizures not having *SCN1A* mutation, suggesting that *SCN9A* could be

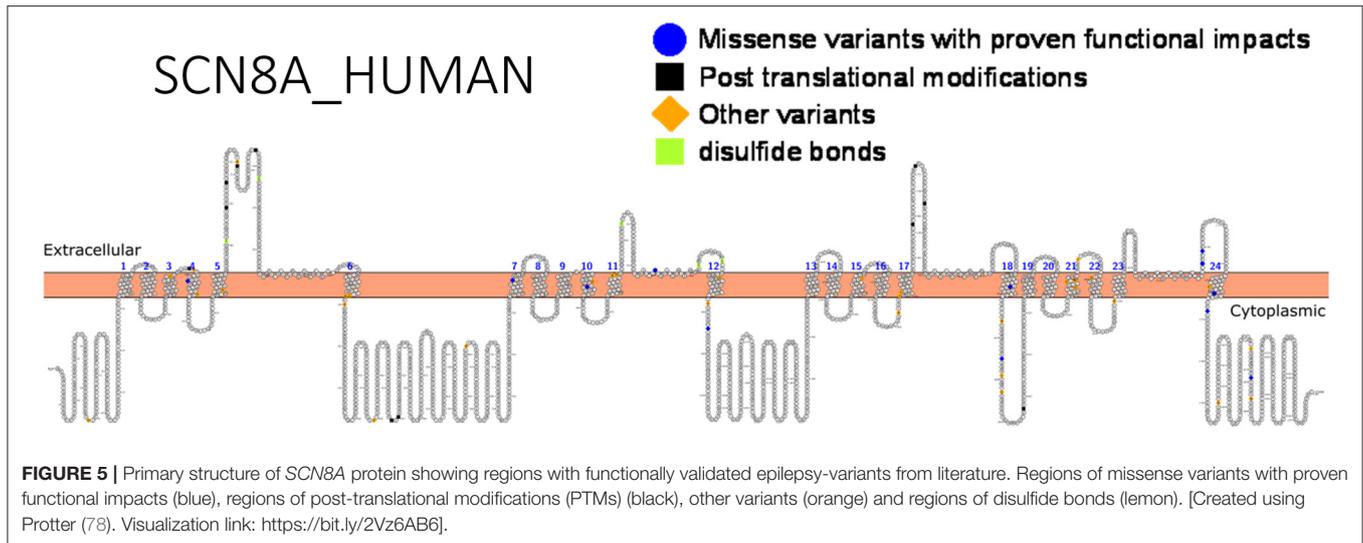


TABLE 7 | Regions of *SCN8A* with Functionally validated epilepsy variants with references.

Gene	Amino acids affected by missense variants	Deletion regions	Protein-truncating variant regions	References
<i>SCN8A</i>	R850, N984, P1719, N1716, G1451, V929, T767, N1768, R223, R1872, G1475, M1760	-	Pro1719ArgfsX6	(100, 138, 140, 141, 144, 148, 153, 154)

TABLE 8 | GWAS reports of sodium channel genes involved in epilepsy.

Gene	SNPID	Associated phenotype	Population studied	References
<i>SCN1A</i> , <i>SCN1A-AS1</i>	Rs7587026-A	Mesial temporal lobe epilepsy with hippocampal sclerosis	Europeans	(160)
<i>SCN1A</i> , <i>SCN1A-AS1</i>	rs11890028-T	Generalized epilepsy	European	(161)
<i>SCN1A</i>	rs6732655-T	All epilepsy	European, Australian, United states, Han Chinese	(162)
<i>SCN1A</i>	rs12987787-T	Partial epilepsy	European, Australia, United states, Han Chinese	(162)
<i>SCN1A-AS1</i> , <i>SCN1A</i>	rs6432860-G	Febrile seizure	European	(163)
<i>SCN1A-AS1</i> , <i>SCN1A</i>	undefined	Generalized epilepsy	Europeans and African American	(164)
<i>SCN1A-AS1</i> , <i>SCN1A</i>	rs2212656-A	Partial epilepsy	European, African American, Han Chinese	(164)
<i>SCN1A-AS1</i> , <i>SCN1A</i>	rs6432877-G	All epilepsy	European, African American, Han Chinese	(164)
<i>SCN2A</i>	rs3769955-T	Febrile seizure	European	(163)

a major participant in cryptogenic epilepsies (158). Recently, Zhang et al. (159) discovered a possibly pathogenic missense variant; Y1958C causing GEFS+ in a Chinese family. *In silico* prediction algorithms validated this variant as likely deleterious but functional validation is still required. Due to the sparsity of *SCN9A* mutations in epilepsy, reports classifying it as a true epilepsy gene are inconclusive.

GENOME WIDE ASSOCIATION STUDIES (GWAS) OF SODIUM CHANNEL GENES INVOLVED IN EPILEPSY

Some reports from GWAS of epilepsy in different populations has shown some high-risk susceptibility single nucleotide

polymorphisms (SNPs) within sodium channel genes (Table 8). These include studies of European, Asian, African-American origin.

In spite of the abundance of studies pointing out the roles of sodium channel genetic mutations in epilepsy, results published on the GWAS catalog (<https://www.ebi.ac.uk/gwas/>) are still relatively few. Feenstra et al. (163) conducted a genome wide association studies on the genetic causality of febrile seizures in individuals presenting with vaccine-associated and vaccine-unassociated seizures (163). Genetic loci associated with epilepsy (2q24.3) included two different sodium channel genes (rs6432860 in *SCN1A* and rs3769955 in *SCN2A*). More GWA studies in diverse population have confirmed the involvement of the genetic loci, 2q24.3 in both monogenic and polygenic epilepsies. In a cohort study of 8696 cases, the SNP, rs6732655-T was associated

with all epilepsy phenotypes while rs12987787-T was associated with focal epilepsies (162). Both SNPs mapped to *SCN1A* within the 2q24.3 genomic loci.

A larger cohort of 15,212 cases identified 16 genomic loci associated with different epilepsy phenotypes (164). Amongst these was the 2q24.3 locus harboring *SCN1A-SCN3A*. The lead SNPs in this region were rs6432877-G for all epilepsies and rs11890028 (G) for genetic generalized epilepsies. The same locus was previously identified as causality for common generalized epilepsies (161). Very large sample sizes are required to increase power and identify more loci in GWAS of epilepsy. As a complex and genetically heterogeneous disorder, it is also difficult to measure heritability in polygenic epilepsies. Hence, small cohort sizes (e.g., <3,500 cases) may be unable to establish heritability of rare variants with small effect sizes (165). This warrants the adoption of large cohort sizes. It would be advantageous if many countries would adopt the establishment of biobanks with detailed sampling information, as this would minimize sampling time in large cohorts studies (166).

Many SNPs are expected to have mitigated or aggravated effects when they occur in the presence of other gene mutations (i.e., epistatic interactions). Although GWAS reports may give a clue to the presence of pleiotropic effects amongst genes (164), they do not account for the actual damage-potential of the variants under the influence of mutations from other genes. Hence, necessitating functional studies.

Despite the drawbacks of GWAS, its innate capacity still needs to be harnessed. More research collaborations and funding are required to promote GWAS in regions with high epilepsy burden. GWAS of epilepsy in epilepsy endemic regions are expected to discover ethnically related polymorphisms and genetic diversity of epilepsy amongst different populations (167).

CONCLUSION AND FUTURE CONSIDERATIONS

Since epilepsy is a polygenic and heterogeneous disorder, the increasingly cheap cost of whole genome and exome sequencing is rapidly advancing variant identification in candidate genes, including those acting as disease modifiers. The exponential increase in detected variants has created a huge need for more functional studies to validate the underlying mechanisms. Even though the evolutionary conservation amongst the different α -subunits has prevented the designing of drugs specifically for different VGSC channels, some progress has been recorded in determining effective therapies based on the knowledge of molecular mechanisms of mutations. Meanwhile,

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the outcome bias introduced in the functional analyses done using heterologous mammalian cells lines should be rapidly eliminated using patient-derived, genetically engineered neurons from hiPSCs or transgenic animals. Moreover, computational methods should be considered to guide decision-making for appropriate variants for functional studies. Above all, the field of epilepsy genetics has experienced a rapid improvement and much understanding has been gained over the past decades.

LIMITATIONS OF THIS STUDY

Reports reviewed in this article were majorly from European, Asian, and American populations. We observed the absence of genetic studies on epilepsy from African populations, despite the high prevalence of this disorder in Africa. Fodjo et al. (168) reported the prevalence of epilepsy in West Africa as 13.14 per 1,000 persons, Akinsulore and Adewuya (169) reported an incidence rate of 5.3–37 per 100 persons in Nigeria, Esterhuizen et al. (167) reported a median occurrence of 14.2 per 1,000 in Sub-Saharan Africa while Mukuku et al. (170) reported 5.2–74 per 100 persons with epilepsy. Although variances occur depending on the studied region, there is remarkably higher prevalence of epilepsy in low- and middle-income countries (LMICs) than in developed nations (171). Over three-fourths of persons having epilepsy live in the LMICs, and about two-thirds of these cases have unknown etiology (167, 170). Although some reports suggest a relationship between epilepsy and CNS infections in Africa (168, 172), the etiology of most epilepsy remains unknown. This emphasizes the role of genetics and an urgent need for genetic investigations.

AUTHOR CONTRIBUTIONS

AI, RS, and AE developed the concept of the manuscript. AI wrote the first draft. RS, AE, AY, and SS reviewed and modified the draft. All authors approved the final version of the work.

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