REVIEW

The SCN1A gene variants and epileptic encephalopathies

Rashmi Parihar^{1,2} and Subramaniam Ganesh¹

The voltage-gated sodium channels are fundamental units that evoke the action potential in excitable cells such as neurons. These channels are integral membrane proteins typically consisting of one α -subunit, which forms the larger central pore of the channel, and two smaller auxiliary β -subunits, which modulate the channel functions. Genetic alterations in the *SCN1A* gene coding for the α -subunit of the neuronal voltage-gated sodium ion channel, type 1 (Nav 1.1), is associated with a spectrum of seizure-related disorders in human, ranging from a relatively milder form of febrile seizures to a more severe epileptic condition known as the Dravet syndrome. Among the epilepsy genes, the *SCN1A* gene perhaps known to have the largest number of disease-associated alleles. Here we present a meta-analysis on the *SCN1A* gene variants and provide comprehensive information on epilepsy-associated gene variants, their frequency, the predicted effect on the protein, the ethnicity of the affected along with the inheritance pattern and the associated epileptic phenotype. We also summarize our current understanding on the pathophysiology of the *SCN1A* gene defects, disease mechanism, genetic modifiers and their clinical and diagnostic relevance.

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INTRODUCTION

Among the genes known to be involved in epilepsy, the *SCN1A* gene represents one of the most commonly mutated human epilepsy genes, referred to be as super culprit gene,¹ Approximately 900 distinct mutations reported in close to as many families. The *SCN1A* gene codes for the α -subunit of the neuronal voltage-gated sodium ion channel, type1 (Nav 1.1),² and is expressed in the central and peripheral nervous systems and in cardiac myocytes.^{1,3,4} Mutations in the *SCN1A* gene were first reported in two families with generalized epilepsy with febrile seizures plus (GEFS+),⁵ although subsequent studies have shown that a majority (>80%) of the mutations are associated with the Dravet syndrome (DS) or its variants called severe myoclonic epilepsy, borderline (SMEB)^{6,7} and intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC).⁸ Both of these latter cases represent a phenotype lacking one or more features of the classical DS.⁹ Therefore, the *SCN1A* gene mutations are thought to represent a genetic form of early-onset epileptic encephalopathies¹⁰ with a common designation as DS¹¹ (MIM#607208). Besides DS and its variants, *SCN1A* mutations are also associated with other rarer forms of epileptic encephalopathies as listed in the Table 1. As DS spectrum disorders account for the majority of the reported cases with the *SCN1A* gene mutations, we restricted our analyses on DS and tried to offer as much information as possible to the readers on DS.

DS, also known as severe myoclonic epilepsy in infancy (SMEI) (MIM# 607208), is an intractable childhood-onset epileptic encephalopathy, first reported by Charlotte Dravet, a French epileptologist.¹² DS is characterized by the prolonged febrile hemiclonic or generalized tonic, clonic and tonic-clonic seizures accompanied by fever during the first year of life.^{13–16} DS patients also show a delay in psychomotor development, dysphasia, ataxia and other neuropsychological deficits from the second year of life.¹⁷ The other seizure types seen in DS patients include myoclonus, absence and partial seizures,¹⁸ and almost all seizure types are remarkably resistant to anti-epileptic medications.¹⁹ The incidence rate of DS is nearly one in around 30 000 live births^{20,21} and more than 85% of the DS patients survive up to adulthood,^{22–24} although early death in infancy is also known in several cases.^{11,25} Curiously, males are more often affected with DS than females, with the ratio of 2:1, however, a reason for this skewed ratio is unknown.^{20,21}

DS is caused by *de novo* heterozygous mutations in the *SCN1A* gene in about 95% of the patients^{26,27} (see Supplementary Table 1). The *SCN1A* gene is located on the chromosome 2q24, composed of 26 exons spanning 6030 bp and encodes the large α -subunit of the voltage-gated sodium ion channel, type 1 (Nav 1.1)²⁸ (see Figure 1). Additional alternate exons harboring the 5'-untranslated region of the gene have been reported, but the exact combination of these with the coding exons is yet to be resolved.²⁹ Alternate splicing of exon 5 of

¹Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur, India and ²Department of Biochemistry, G.R. Medical College, Gwalior, India
Correspondence: Professor S Ganesh, Department of Biological Sciences & Bioengineering, Indian Institute of Technology, Academic Area, Kanpur 208016, India.
E-mail: sganesh@iitk.ac.in

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Table 1 Epileptic encephalopathies associated with variations in the *SCN1A* gene

Epileptic encephalopathy	Clinical features	References
SMEI or DS	Prolonged febrile hemiclonic or generalized tonic, clonic and tonic-clonic seizures accompanied with the fever during the first year of life	13,26
SMEB	Lacking one or more features of SMEI-like myoclonic seizures or generalized spike and wave activity	18,107
FS	Childhood febrile convulsions usually associated with high fever.	7
GEFS +	Begin between infancy and 3 years of age; febrile seizures followed by episodic tonic, clonic, myoclonic or absence seizures.	5
ICEGTC	Childhood epilepsy with generalized clonic, generalized tonic clonic seizures which are intractable, normally myoclonic and absence seizures are not present	8,108
CGE	Characterized by 'multiple seizure types, generalized sharp and slow activity and intellectual disability with no known etiology'	17
CFE	Characterized by 'focal seizures and uni- or multifocal EEG epileptiform patterns'	17
MAE or Doose syndrome	Characterized by 'myoclonic-astatic seizures and other generalized seizure types with generalized spike-wave activity and variable developmental outcome'	17,109,110
SIGE1	Epilepsy of infancy characterized by febrile and afebrile generalized tonic-clonic seizures as the only seizure type.	110
RE	A progressive inflammatory encephalopathy characterized by refractory partial seizures and cognitive decline due to gradual atrophy of a single brain hemisphere.	111–113
IS or WS	An infantile epileptic encephalopathy characterized by infantile spasms, hypsarrhythmia and mental retardation.	114,115
LGS	A severe form of early childhood epilepsy characterized by tonic, atonic, myoclonic and atypical absence seizures and cognitive/behavioral impairment.	17,116–118

Abbreviations: CFE, cryptogenic focal epilepsy; CGE, cryptogenic generalized epilepsy; DS, Dravet Syndrome; FS, febrile seizure; GEFS +, generalized epilepsy with febrile seizure, plus; ICEGTC, intractable childhood epilepsy with generalized tonic-clonic seizures; IS, infantile spasms; LGS, Lennox-Gastaut syndrome; MAE, myoclonic astatic Epilepsy; RE, Rasmussen's encephalitis; SIGE1, severe idiopathic generalized epilepsy of infancy; SMEB, severe myoclonic epilepsy in infancy—borderline; SMEI, severe myoclonic epilepsy in infancy; WS, West syndrome.

the *SCN1A* gene is known to give rise to two alternate exons (exon 5N and 5A, expressed in neonates and in adults, respectively), and it has been suggested that duplication events could have lead to the evolution of such mutually exclusive exons in independent lineages.³⁰ As expected of its function, in rodents, the expression of the *Scn1a* gene becomes noticeable post partum and it increases till the adulthood, predominantly in the caudal region and the spinal cord.^{31,32} Within the CNS, *Scn1a* shows higher expression in dendrites, cell bodies^{31,33} and also at the parvalbumin positive inter-neuronal axon initial segments.^{32,34} The *SCN1A*-coded α -subunit is a 260 kDa protein that harbors four homologous domains, each spanning six transmembrane segments.³⁵ The NaV 1.1 channel is heteromeric complex consisting of the α -subunit, which forms the larger central pore of the channel, and two smaller auxiliary β -subunits.³⁶ The α -subunit regulates the sodium ion selectivity and can on its own function as a channel,^{35,37,38} whereas β -subunits regulate the gating and kinetics of the channel and also help in the interaction with the cytoskeleton and other proteins for the trafficking of the channel protein to the cell membrane.^{2,35,39,40}

Approximately 700 distinct sequence variations in the *SCN1A* gene are known to be associated with the DS (Figure 1; Supplementary Table 1) and all of them were found in heterozygous state, suggesting that their homozygous state could be embryonic lethal. These variations were found all along the coding region of the gene and also include large deletions and duplications (Supplementary Figure 2; Supplementary Table 1. Therefore, it can be proposed that these disease-associated variations are the loss-of-function mutations and the DS results from haploinsufficiency.^{38,41–44} A support for this notion comes from animal models wherein both homozygous and heterozygous *Scn1a* knockout mice developed epileptic seizures soon after birth.⁴⁵ More importantly, a knock-in mouse model having a common DS-associated *SCN1A* mutation developed seizure phenotype both in homozygous and heterozygous states.³² Thus, a significant reduction in the sodium channel activity is likely to underlie the seizures phenotype⁴² (also see below). These animal models have shown that sodium channels are essential for the survival, as *Scn1a* null mutants exhibited premature death.⁴⁶

Functional studies, especially the electrophysiological approaches, have suggested that the possible 'gain-of-function' effect for some of the disease-associated *SCN1A* variants and offered an alternative hypothesis on the etiology of the *SCN1A*-associated diseases.^{47–56}

THE SPECTRUM OF DISEASE-ASSOCIATED *SCN1A* GENE VARIANTS

Among the epilepsy genes, the *SCN1A* gene perhaps is known to have the largest number of disease-associated variants so far, and therefore the *SCN1A* gene is also rightly named as 'super culprit gene'.¹ Two databases on the *SCN1A* gene mutations are reported in the literature; the *SCN1A* Infobase¹ (<http://www.scn1a.info/>) and the *SCN1A* variant database⁵⁷ (<http://www.molgen.ua.ac.be/SCN1AMutations/>). However, both of them have not been updated for the last couple of years, and these two databases do not provide important information such as the number of cases reported for each gene variant, the population of origin and the tested/predicted effect of the reported missense mutations. We therefore felt the need to create an up-to-date list of all the *SCN1A* gene variants reported to be linked with the DS-related phenotype. For this, we carried out a thorough review of the scientific literatures published in the English journals that reported a causal relationship between *SCN1A* gene and the epilepsy phenotype. For this, we have searched the PubMed and Medline using the query words *SCN1A* and SMEI, DS, SMEB or GEFS +. The search included all the papers that are listed in the PubMed until January 2013. Not all reports mention the ethnicity of the patients with the *SCN1A* gene defects; we therefore requested to the authors of such papers to provide us the ethnicity details of the patients in their report, and we included the details in the mutation list if such information was made available (names of authors who have kindly responded to our requests are listed in the acknowledgment section). Thus, the comprehensive list of mutations created, given in the Supplementary Table 1, was manually curated for the mutation and its frequency, its predicted effect on the protein, the ethnicity of the affected, the inheritance status of the mutation and the associated epileptic phenotype. We followed the guidelines of Human Genome Organisation (www.hgvs.org/mutnomen) for the mutation nomenclature;

Figure 1 Schematic diagram of the *SCN1A* gene showing coding exons in gray boxes and introns as black lines (not in scale). The position (arrows) and frequency of various *SCN1A* mutations (present in two or more patients, shown as bar diagram) are shown. Larger deletions and duplications are listed on the top left. A complete list of mutations and their source are given in the Supplementary Table 1. The mutation nomenclature used in the schematic is based on GenBank protein sequence reference number NP_001159435.1, and as suggested by the Human Genome Variation Society.¹⁰⁶

nucleotide 1 is the A of the ATG-translation initiation codon of the RefSeqGene (NG_011906.1) for the *SCN1A* gene. This RefSeqGene lists 26 exons, and therefore the exon designation given in the Supplementary Table 1 is as per this sequence. The four 5'-end untranslated exons of the *SCN1A* gene, reported by Long and his colleagues,^{29,58} are identified in the Supplementary Table 1 with the

designation as reported in the original publication (h1u, h1a, h1b and h1c). All mutations were doubly checked for accuracy. As the human *SCN1A* gene is known to code for at least three distinct transcripts,⁵⁹ there is no uniformity in the literature with regard to which transcript should be used as the reference set for numbering the *SCN1A* gene mutations. Therefore, the reported mutations cannot be directly

compared. In this report, we reanalyzed the mutations, and if the original report used the sequence of a shorter transcript of the *SCN1A* gene for numbering then the same has been corrected on the basis of the RefSeqGene (NG_011906.1; which represent the longest transcript) and renumbered accordingly in our data set (see Supplementary Table 1). For the missense mutations, we have also looked at the phylogenetic conservation of the affected residue and listed the same (see Supplementary Table 2). For this, the individual protein sequence of the α -subunit of the NaV 1.1 channel of *Homo sapiens* (NP_001159436.1), *Mus musculus* (NP_061203.2), *Rattus norvegicus* (AAA79965.1), *Bos taurus* (NP_001180147.1), *Canis lupus familiaris* (XP_535941.2) and *Equus caballus* (XP_001916728.2) were downloaded from the NCBI and aligned using the ClustalW program (see Supplementary Figure 4). Thus, this mutation update perhaps represents one of the most comprehensive analyses on the *SCN1A* gene variants associated with the epilepsy.

A total of 786 *SCN1A* gene variants are reported to be associated with DS (682), SMEB (69) or the GEFS+ (35) (see Supplementary Table 1 and Supplementary Figure 1). Therefore, DS accounts for the majority of the mutations (86%) known in the *SCN1A* gene. It may be noted here that the *SCN1A* gene variations are exclusive to the each group of epilepsy; of the 786 variants in *SCN1A*, only 27 of them are known to be associated with both DS and SMEB and only six with both DS and GEFS+ (see Supplementary Figure 1). Similarly, only two variants associate with both SMEB and GEFS+ and only one variant associates with all the three phenotypes (see Supplementary Figure 1), suggesting that the majority of the *SCN1A* gene variants are unique to the three phenotypes discussed above. At the protein level, missense mutations account for about 45% of the known variants in the *SCN1A* gene (Figure 2), comparing well with the data reported in the Human Gene Mutation Database (HGMD)⁶⁰ (<http://www.hgmd.org>). Similarly, nonsense mutations (11%), small deletions (16%), splice mutations (11%) and large deletions (4.3%) in *SCN1A* are also in the same frequency as that reported in the HGMD. However, INDELs appear to be less frequent in the *SCN1A* gene (1.6%) as compared with the 15% occurrence reported in HGMD, although its significance is not obvious to us. It should be

noted here that the mutations in *SCN1A* are *de novo* in origin in the majority of the patients (88%), and only 12% of the affected inherited the mutation from one of the parents (see Supplementary Table 1). Interestingly, several studies have looked at the parent of origin for the mutant allele—both for the inherited and *de novo* forms—and the observed patterns were strikingly opposite. Among the familial cases, 78% of the affected (39 out of 50 cases) received the mutant allele from their mother (see Supplementary Table 1), whereas in the *de novo* events the allele that is mutated more often appears to be paternal in origin (~75%, in 46 out of 61 cases, see Supplementary Table 1).^{61–63} Although a possible cause for the skewed distribution in the familial cases is not obvious, it has been suggested that the differences in the gametogenic process between the human male and female could possibly be the reason for the increased incidence of the paternal allele undergoing mutation in *de novo* cases.⁶³ For example, the meiotic entry in all egg cells is complete before birth in case of females, whereas in males spermatogenesis is a continuous process; further, the germ cells in males can more likely be exposed to mutagenic triggers, and hence more often the paternal allele could bear the mutation and appear as a *de novo* event.⁶⁴

As shown in Figure 1, genetic alterations in the *SCN1A* gene associating with the epileptic phenotype are seen through the gene sequence. Although genetic variants affecting the coding region of the *SCN1A* gene appear to be far more frequent, possibly due to the bias in the approach that normally restricts the analysis to the alterations in the coding region, sequence variations affecting the regulatory regions of the gene have also been reported. For example, detection of genomic deletion occurring at the 5'-end of the *SCN1A* gene led to the characterization of two promoter elements of the gene.⁶⁵ Given that a majority of the mutational events in the *SCN1A* gene appear to be *de novo* in origin, it is intriguing to note that the most frequent mutational event is the large deletion removing the entire *SCN1A* gene (see Figure 1). Nonsense mutations p.Arg222* (15 cases) and p.Arg712* (13 cases) are the other two common mutations (Figure 1), and both of them resulted from the C-to-T transition at CpG sites, susceptible to deamination of methylated cytosine (see Supplementary Table 1). Our data set also allowed us to test whether the reported gene variants show any correlation with the ethnicity of the patients. As shown in the Supplementary Figure 3, the two groups (Asian origin and European origin) of patients did not show any difference in the frequency of the gene variants.

A total of 399 missense mutations are known in *SCN1A*, out of which 395 affect a conserved residue in the predicted protein (based on our ClustalW alignment; see Supplementary Figure 4), suggesting that each of these residues is critical for the function of the encoded protein (see Supplementary Table 2). This notion is reinforced by the observation that a significant proportion of the missense mutations (29%) are clustered at P-loop region of channel, which is responsible for the ion selectivity, and in the intracellular amino- and carboxyl-termini (12%), which modulate the activation/inactivation of channel⁶⁶ (Figure 2). The remaining mutations (59%) were found in the 20 trans-membrane segments of the channel. Most of these mutations are expected to severely impair the channel function.⁶⁷ Moreover, functional studies have indicated that indeed this could be the case for a few of those tested. For example, some of the mutants, when tested in cellular models, showed reduced cell surface expression, suggesting that impaired trafficking of the channel complex to the plasma membrane might underlie the disease.⁶⁸ Electrophysiological properties for some of these mutant channels were also tested and were found to show mixed biophysical properties; for example, whereas some mutants were

Figure 2 The schematic diagram showing the domain organization of the α -subunit of the voltage-gated sodium ion channel coded by the *SCN1A* gene and the positions of the missense mutations (shown as black circles). The α -subunit consists of four homologous domains (D1–D4), each having six trans-membrane segments (numbered S1–S6). The P-loop region of the channel, located between S5–S6, is identified with an asterisk. For details of individual mutation, see Supplementary Table 2.

found to be 'non functional', a few other mutants showed persistent inward sodium current, possibly indicating a gain-of-function abnormality^{47,49,59}; a few, however, did not show any difference in channel activity as compared with the wild-type channel.^{47,49} Thus, although the phenotypic variations seen among the patients could be due to the difference in the electrophysiological properties of the mutant channel, the disease *per se* may result from more than a single molecular pathway, and the aberrant channel activity could be one of them.

THE SCN1A GENE VARIANTS AND PHENOTYPE CORRELATIONS

The general consensus has been that the *SCN1A* gene mutations cause a spectrum of phenotypes, ranging from the severe classic form of the DS to less severe forms in the order SMEB, GEFS+ and febrile seizure—the mildest form.⁶⁷ Thus the channel functions in DS patients are expected to be more severely affected than those, for example, with the GEFS+ phenotype. Consistent with this model, we find that the mutations causing truncation of the *SCN1A* protein are more frequent in patients with DS rather than those that show SMEB or GEFS+ phenotype (see Supplementary Figure 5), suggesting that less severe genetic alterations could possibly be associated with GEFS+. This notion was supported by the observation that the missense mutations are more frequent in the GEFS+ group and least in the DS (see Supplementary Figure 5), and that the *de novo* mutations are more frequent in DS and SMEB than in GEFS+, whereas inherited mutations are more frequent in GEFS+ (see Supplementary Figure 6). Even among the missense mutations, mutations affecting the voltage and/or ion-pore regions were found to be greater in DS than in SMEB or GEFS+. ⁶⁷ Such predictions were also proven to be true for limited set of mutations using bioinformatics analysis⁶⁹ or electrophysiological assays.⁷⁰ However, no positive correlation has been found in between a specific mutation and a specific phenotype.^{67,71} Also, the findings that the same gene alteration could result in either the DS or SMEB, and the same mutation could show variable phenotype among family members when inherited in a family suggest the possible involvement of modifiers of the phenotype. For example, the *SCN1A* gene variants are not fully penetrant as patients with the same genetic alterations are known to show a broad spectrum of seizure phenotypes.⁷² Guerrini *et al.*⁷³ and Singh *et al.*⁷⁴ have independently shown that an independent mutation in the *SCN9A* gene modulates the disease severity of patients with DS and familial mutations in the *SCN1A* gene. In one of these two reports,⁷³ a large deletion in the *SCN1A* gene appears to confer a milder phenotype in the absence of a modifying allele in the *SCN9A* gene coding for another form of a sodium channel, suggesting that the penetrance of *SCN1A*-associated DS is dependent on other genetic factors, and that *SCN1A* gene screening should not be avoided in patients with milder phenotype. Similarly, allelic variants of the *CACNA1A* gene, which encodes a subunit of a voltage-dependent calcium channel, were also shown to modulate epileptic symptoms in DS cases with *SCN1A* mutation.⁷⁵ Although a physiological link between these modifying alleles and the *SCN1A* variations is yet to be established, these observations nonetheless provide an insight into the complex interactions between ion channels in the epileptic phenotype and on the possible avenues for intervention strategies. Supporting this notion, mutations in other genes have been identified in DS patients who did not carry any alterations in the *SCN1A* gene. Examples for such genes include *SCN1B*,^{76,77} *SCN2A*,^{47,71,118} *PCDH19*^{78,79} and *GABRG2*.^{80,81}

Obviously, detailed functional studies are required to understand the molecular basis of genetic modifiers in the DS.

ANIMAL MODELS WITH THE *Scn1a* GENE DEFECTS

Much of our understanding on the mechanism by which *SCN1A* mutations could result in epilepsy has come from the animal models for the DS, created by deleting the *Scn1a* gene in mice or by replacing the wild-type mouse gene with a disease-associated mutant version analogous to one often seen in human DS patients.^{32,45,74,82–86} Mice homozygous for the *Scn1a* null mutation developed severe ataxia and seizures, and died on day 15 post partum.⁴⁵ Mice heterozygous for the gene mutation exhibited spontaneous seizures, and the spontaneous death occurred on or after day 21 post partum. Curiously, some of these phenotypes were dependent on the genetic background, suggesting the role of genetic modifiers in the disease severity.⁴⁵ A knock-in mice model, wherein the murine *Scn1a* gene was altered to represent a human allele harboring a nonsense mutation in the *SCN1A* gene, also developed epileptic seizures within a month after birth—both in heterozygous and in homozygous conditions³²—suggesting that the functional loss of the *SCN1A* gene could result in the epileptic phenotype as seen in the DS. In both of these models, the physiological dysfunctions in the interneurons (neurons that form local circuits between other two neurons) were thought to underlie the epileptic seizures.^{32,45} This notion was supported by the observation that the conditional knockout of *Scn1a* gene only in the inhibitory interneuron in mouse resulted in seizure and premature death,⁴⁶ suggesting that interneurons have a critical role in the pathogenesis of *Scn1a*-associated epilepsies. As expected, the *Scn1a* gene is expressed in inhibitory interneurons^{32,87} and the deletion of the *Scn1a* gene impairs sodium ion currents and evoked action potentials specifically in the GABAergic interneurons, wherein NaV 1.1 represents the primary sodium channel.^{46,86,88,89} Similar observations were also made in a transgenic mice model, wherein a GEFS+-specific *SCN1A* gene mutation was expressed in *Scn1a* knockout mice.⁸⁵ Intriguingly, the GEFS+-specific transgenic channel was able to partially rescue the survival of *Scn1a* knockout mice,⁸⁵ suggesting that the GEFS+-specific mutation results in the partial loss of the channel function, and therefore the GEFS+ is a milder phenotype as compared with the DS. A recent report that looked at the mechanism behind the early death in the *Scn1a* knockout mice demonstrates that parasympathetic hyperactivity following the tonic-clonic seizures results in the lethal bradycardia.⁹⁰ One of the intriguing observations reported in the animals for DS is the behavioral deficits, such as hyperactivity, stereotyped behaviors and poor social interaction.^{88,91} These autism-spectrum behaviors were also seen in patients with the DS,⁹² although it was not evident whether the genesis of these abnormal behaviors is secondary to the seizure-induced neuronal injury or a direct cause of the *Scn1a* gene loss. A recent study demonstrates that a low dose of benzodiazepine clonazepam—a positive modulator of GABAA receptors—corrected the behavioral abnormalities in mice heterozygous for the *Scn1a* gene mutant,⁸⁸ suggesting that the autism-spectrum behaviors could be due to the defects in the *SCN1A*-dependent GABAergic signaling cascade. Similar to the observations made in human DS patients, the mice models for DS also showed effect of genetic modifiers; the null for the *Scn1a* gene in the 129/SvJ strain background showed fewer incidences of spontaneous seizures and survived longer as compared with the same allele in the C57BL/6J strain background.⁴⁵ Genetic interactions between the *Scn1a* gene and the potassium channel gene *Kcnq2* and also between the *Scn1a* gene and the *Scn8a* gene have been reported to

modify the seizure phenotype in DS mice models.^{83,93} Thus, these animal models could be used for the discovery of novel genetic modifiers for the DS. On similar note, one of the recent studies on the DS mice model suggests that ketogenic diet might provide protective effect on refractory patients with *SCN1A* mutations.⁸⁷ Taken together, studies on the genetically engineered animal models for DS provide a mechanistic insight into the epileptic seizures, early death and behavioral deficits and also these models might help in discovering novel treatment strategies.

THE *SCN1A* GENE VARIATIONS: CLINICAL AND DIAGNOSTIC RELEVANCE

The NCBI GeneTests website (<http://www.ncbi.nlm.nih.gov/sites/Genetests/>) provides a list of clinical laboratories, along with the contact details, that carry out the genetic screening for the DS. Considering the allelic heterogeneity in the *SCN1A* gene variations, sequencing the entire gene sequence (including the regulatory regions) seems to be the first choice for the genetic screen. This seems to be the ideal approach as there are no clear-cut mutational hotspots for the *SCN1A* gene and a majority of the disease-associated genetic alterations seen in the *SCN1A* gene that are *de novo* in origin, and therefore the founder effect has negligible role in the disease occurrence. The large duplication and deletion mutations in the *SCN1A* gene associated with DS pose another challenge in the routine diagnosis; such mutations escape detection when conventional PCR amplification and DNA sequencing are attempted, and the copy-number variation can only be detected when multiplex ligation-dependent probe amplification assay or the comparative genome hybridization arrays were used.⁹⁴ Thus, absence of 'detectable' mutation in the conventional sequencing approach should be interpreted with caution.⁸⁰ Moreover, one could look for copy-number variation in the *SCN1A* gene in such patients because the frequency of deletions/duplications might be more than what is being calculated as of now. Given that the clinical spectrum of pediatric epilepsy, a few reports suggest that the genetic screen for the *SCN1A* gene mutations may be recommended to only those infants who meet a set of clinical characteristics.^{7,95} For example, patients having the onset of febrile seizure at 7 months age or earlier, and those with frequent (>5/day) and prolonged (>10 min) seizures are more likely to have mutations in *SCN1A* gene as compared with those with a milder clinical form.^{7,96} Another report suggests that normal developed before the onset of seizure and exacerbation with hyperthermia is a good predictor of the *SCN1A* mutation in DS cases.⁹⁶ In each of these cases, a thorough clinical diagnosis appears to be a pre-requisite to recommend a case for DNA screening. To overcome some of these limitations, the use of next generation sequencing methods have been advocated for the genetic screens for epileptic patients. Lemke *et al.*⁹⁷ have reported a cost-effective method wherein possible mutations in 265 genes, which includes the *SCN1A* gene, were analyzed for each patient in a single-step process, and this method could be used to diagnose the genetic basis even in clinically unresolved cases. This approach appears to be a better option, as even patients with deletion in the *SCN1A* gene may show milder forms of seizure as the severity could be modulated by genetic modifiers.⁷³ Parental mosaicisms were also reported in the DS.^{63,98} Thus, mutational screening could help expectant couples to consider prenatal diagnosis. Finally, there is no consensus with regard to the usefulness of the genetic screening in treatment modalities, as the knowledge of genetic basis of epilepsies did not seem to significantly improve the treatment modalities.⁹⁹ A few reports suggest that sodium channel blockers such as carbamazepine and lamotrigine should be avoided as anti-

convulsant, as they may aggravate seizure in *SCN1A* patients.^{99,100} Similarly, *SCN1A* genetic diagnosis was reported to show positive influence on the choice of the medication and was shown to improve seizure control especially for the younger DS patients.¹⁰¹ An intronic single-nucleotide polymorphism (SNP; rs3812718) in the *SCN1A* gene was shown to serve as 'predictor' for the use of drugs (phenytoin and carbamazepine) at maximum dose with minimal side effects.^{81,102,103} The *SCN1A* gene is known to undergo alternate splicing, and this SNP affects the consensus sequence for the 5'-splice donor site of exon 5 that undergo alternative splicing to produce distinct *SCN1A* splice variants.¹⁰² As a result, the relative proportion of the splice *SCN1A* variants in the neonate and adult brain tissues was shown to be altered in individuals that carry the SNP.¹⁰³ An association for this SNP in the drug response, however, could not be replicated in all populations.^{104,105} Thus, such studies should be extended to other populations and a large prospective multi-centre cohort study is exceedingly essential. Such genetic screens could ensure accurate diagnosis of DS, to help genetic counseling and patient/family management, and eventually in improving treatment modalities.

CONCLUSION AND FUTURE PROSPECTS

The DS spectrum disorders are considered as rarer forms of genetic encephalopathies with epileptic seizures as the major symptom. Despite its rarity, a large number of studies have looked at the genetic cause of DS and relatively a large body of data is available in the public domain with regard to the prevalence of mutations in the *SCN1A* gene and the phenotype correlates. These advances, however, did not significantly alter the treatment modalities or its outcome. Therefore, it is important to elucidate the physiological effects of the *SCN1A* gene variants and to understand the disease mechanisms. The animal models for the *Scn1a* mutations are invaluable resources to understand the pathomechanisms and in the development of therapeutic protocols.

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