RESEARCH PAPER

SCN1A Gene Mutations in Indian Children With Epilepsy: Single Center Experience

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Objective: To study prevalence of *SCN1A* gene mutations in complex seizure disorders. **Methods**: Retrospective laboratory based study on samples sent for molecular diagnosis in complex seizure disorders. Exome sequencing was performed. Phenotype- genotype correlation was done for patients showing variants in *SCN1A* gene. **Results**: 364 samples were evaluated; of which, 54% were of children below 5 years of age. *SCN1A* mutations were seen in 50 samples of patients with complex seizure disorders; 44 variants were identified. Types of seizure disorders commonly associated were Dravet syndrome and genetic epilepsy with febrile seizures. **Conclusions**: *SCN1A* mutations are common in complex seizure disorders, especially Dravet syndrome. Early identification of *SCN1A* gene in etiology is important for selection of correct antiepileptic and counselling.

Keywords: *Dravet syndrome, Epileptic encephalopathy, Evaluation.*

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SCN1A is the most frequently mutated gene causing epilepsy and is named as the super culprit gene [1]. It codes for alpha subunit of neuronal voltage gated sodium ion channel type 1, located in chromosome 2q24.3. It is expressed in central and peripheral nervous system. The SCN1A protein controls the flow of sodium ions and thereby regulates the neuronal excitability [2].

More than 1500 pathogenic and likely pathogenic mutations have been reported in *SCN1A* gene causing various epilepsy syndromes. Though *SCN1A* mutations are encountered in milder seizure disorders like simple febrile seizures and generalized epilepsy with febrile seizures plus (GEFS+), identifying mutations of this gene is important in epilepsy disorders with poor cognitive outcome like Dravet syndrome. Inheritance of *SCN1A* related seizure disorders is autosomal dominant. 80% mutations being denovo (spontaneous) mutations. Such denovo mutations are seen in majority cases of Dravet syndrome. Mutations located within the pore and voltage gated channels cause more severe phenotype [2]. Study of *SCN1A* gene mutations with parental segregation can predict clinical course. We report the mutation spectrum of *SCN1A* in Indian patients with seizure disorders.

METHODS

This study involved review of laboratory records of 364 patients whose samples were sent for genetic evaluation, due to presentation with various types of epilepsy. Study was approved by the institutional ethics committee of our center. Peripheral venous blood was collected after informed consent from the patients/guardians, in case the patient was a minor. DNA was isolated using QIAamp DNA blood mini kit (Qiagen Pvt Ltd) following the manufacturer's protocol.

Whole exome sequencing and analysis: Library preparation consisting of pre-capture, hybridization, capture and post-capture was done using Twist Human Core Exome kit by following the manufacturer's protocol. The next-generation sequencing was performed on Novaseq 6000 (Illumina). Sequence reads were analyzed and aligned to the human reference genome (hg19) using Illumina Dragen software. Variants were annotated using VarSeq (Golden Helix Inc.) [3] with *i*) functional consequence in RefSeq gene transcripts, *ii*) zygosity, *iii*) minor allele frequency (MAF) determined using databases (gnomAD, 1000 Genomes) [4], and *iv*) reported in ClinVar [5]. Average depth and coverage of *SCN1A* gene was >90 and 100%, respectively. Data are presented as mean (SD) or proportions.

RESULTS

Of the 364 samples, 50 patients (31 boys) had mutations in *SCN1A* gene. Mean age was 5.5 years. 54% of the patients were below 5 years at diagnosis while 76% were below 10 years of age.

Out of the mutations identified, 31 (62%) had pathogenic or likely pathogenic mutations. The remaining 38% patients had variants of unknown significance in *SCN1A* gene as they

WHAT THIS STUDY ADDS?

- *SCN1A* mutations were found to be common in referred children with complex seizure disorders.

were not reported as pathogenic in ClinVar, and they were either novel or present with very low allele frequency in 1000 Genomes and gnom AD databases. Insilico prediction tools like SIFT/PolyPhen/Mutation taster predicted the mutations to be deleterious and conservation tools like GERP++/ PhyloP predicted the regions to be conserved across mammals. All of the mutations were present either in the ion transport domain or pore region of the protein.

Forty four heterozygous variants in *SCN1A* gene were observed in fifty subjects (**Table I**), comprising 25 missense, eight nonsense, three splice site, five frameshift mutations, one small base pair indel variant, one in-frame deletion, and 1 copy number variant. Two patients had p.Arg1928His variant, three had p.Ser573Arg variant, two had common indel variant (c.1423_1424delinsCT), and three patients had missense variant p.Arg101Trp (**Web Table I**).

DISCUSSION

SCN1A is an important channelopathy gene involved in many epilepsy syndromes. It is a causative gene for Dravet syndrome in majority of the patients, with studies quoting its frequency as high as 90%. Frequency of *SCN1A* mutations in simple febrile convulsions and febrile seizures plus is unknown. In generalized epilepsy with febrile seizures, frequency of *SCN1A* mutation is 5 to 10% [6,7].

Inheritance of *SCN1A* related epilepsy syndromes is autosomal dominant. Most of the mutations, especially in severe phenotypes, are de novo. *SCN1A* mutations are always encountered in heterozygous condition. Homozygous state results in embryonic lethality [8]. All patients in our present study had mutation in heterozygous state. Severity of phenotype depends on location of mutation in the gene. Pathogenic variants located in voltage-gated region or pore regions result in severe phenotype. While in other regions, even the loss of function variants may exhibit milder phenotype. We noted severe phenotype in all the patients, and the same is reflected in the location of the variants; all the mutations were located either in voltage-gated or pore regions. Out of 786 mutations responsible for Dravet syndrome reported in a review, 86% were present exclusively in Dravet syndrome only, 3.4% were associated with both Dravet syndrome and severe myoclonic epilepsy, borderline and less than 1% for both Dravet syndrome and GEFS+ [2]. Though incomplete penetrance and variable expressivity is reported, variant identification can predict future course and syndrome even at young age.

As most of the pathogenic variants in severe phenotypes arise spontaneously and such variants are absent in parents, couples can be assured regarding the negligible risk of recurrence in future pregnancies. This small risk of recurrence in future pregnancies is likely because of gonadal mosaicism in these few instances. However in case the mutation originates from a parent, 50% chance of recurrence is present [6]. If the seizure disorder in the child is severe option of prenatal diagnosis should thus be discussed with the parents. It is also observed that more severe, the phenotype, more likely that it has originated de novo [6].

When investigating the genetic etiology in a patient with a epilepsy, exome sequencing consisting of multi gene panel for seizure disorders with prime focus on *SCN1A* should be considered. This approach can identify other genes responsible, especially for potentially treatable conditions like, pyridoxine dependent epilepsy, biotinidase deficiency, and glucose transporter 1 deficiency. Even in patients presenting with seizures in early infancy, these studies should be undertaken as treatment can be started early in potentially treatable disorders. In conditions like Dravet syndrome, early diagnosis and proper seizure control can slow down cognitive decline. Certain antiepileptics like carbamazepine, lamotrigine and vigabatrin are contraindicated in *SCN1A* seizure disorders as they may induce or increase myoclonic seizures. Phenytoin is also contraindicated as it can increase seizures and induce choreoathetosis. Acetaminophen should

Table I *SCN1A* Mutation Data in the Study Subjects (*N*=50)

Variable	*No. (%)*
Male	31 (62)
Type of mutations	
Missense	25 (50)
Nonsense	8 (16)
Frameshift	5 (10)
Indel	1 (2)
Splice site	3 (6)
CNV	1 (2)
Classification of variants	
Pathogenic/likely pathogenic	31 (62)
Uncertain significance	19 (38)
Phenotype	
Dravet syndrome	36 (72)
Generalized epilepsy with febrile seizures	10 (20)
Non descript seizures	4 (8)

All values in no. (%).

be used with caution, especially when valproate and topiramate are used in *SCN1A* associated seizure control, as hepatotoxicity is likely with even a small overdose. Thus, identification of *SCN1A* mutation early in the course of disease helps in important therapeutic decisions. Vaccinations may result in seizures in *SCN1A* associated disorders, but these episodes do not alter the course. Fever can be controlled better with longer acting non-steroid anti-inflamamatory drug like Naproxen. Usual immunization schedule can thus be followed [9,10].

Limitations of our study are inclusion of only referred patients, and non-avilability of full clinical details. Patients with uncontrollable seizures with two initial drugs/ unprovoked seizures/seizures along with developmental issues/hemi-convulsions, should be considered for testing for *SCN1A* mutations. Our study found *SCN1A* mutation as a common abnormality in selected patients with severe epilepsy syndromes.

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Note: Additional material related to this study is available with the online version at *www.indianpediatrics.net*

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Web Table I SCN1A Gene Mutations in Samples of Indian Children With Epilepsy

Age	*Sex*	*Location*	*Sequence change*	*Amino Acid change*	*Acmg classification*	*Variant Classification*	*Indications*	*Novelty*
3 y	M	Exon 29	c.5783G>A	p.Arg1928His	PM2, PP2	VOUS	Seizures	Novel
1 mo	F	Exon 26	c.5158A>G	p.Ile1720Val	PM2,PP2,PP3	VOUS	Convulsion from day 2, epileptic encephalopathy.	Novel
2 y	M	Exon 24	c.4570C>T	p.Pro1524Ser	PM2, PP3, PP2	VOUS	Myoclonic Jerks, frequent falls, generalized tonic clonic seizures,	Novel
7 y	M	Exon 27	c.4556C>T	p.Pro1519Leu	BS1, BP6, PM5, PP2, PP3	VOUS	Epileptic attack on day 4 of life	Novel
3 y	M	Exon 29	c.5783G>A	p.Arg1928His	PM2, PP2	VOUS	Growth lag, regression in speech, no eye contact	Novel
3 y	F	Exon 23	c.4427A>C	p.Asn1476Thr	PM2, PP2	VOUS	Epilepsy	Novel
10 y	M	Exon 23	c.4396T>	p.Phe1466Leu	PM1, PM2, PP2, PP3	VOUS	Complex partial seizures with extra temporal, febrile seizures.	Novel
3 y	M	Exon 23	c.3999G>A	p.Met1333Ile	PM2, PP3, PP2	Likely Pathogenic	Epilepsy, mild fever	Novel
8 mo[a]	M	Exon 20	c.3454T>A	p.Ser1152Thr	PM2,PP2, PP3	VOUS	Cluster of seizures with fever, supra-refractory status, ketogenic diabetes, on ventilator support for 1 month, high ammonia and lactate normal	Reported
13 y	M	Exon 14	c.2552A>G	p.Asn851Ser	PM1, PM2, PP2	VOUS	Focal seizures on the right side of the brain.	Novel
8 y	F	Exon 15	c.2164A>C	p.Asn722His	PM2, PP2, PP3	VOUS	Generalized seizures	Novel
6 mo	F	Exon 14	c.2006C>T	p.Pro669Leu	PM2, PP2, PP3	VOUS	Seizures since 4 months of age (uprolling of eyeballs, tightening of limbs, frothing).	Novel
2 y	F	Exon 14	c.1982C>T	p.Thr661Ile	PM2, PP2, PP3	VOUS	Seizures	Novel
9 y	F	Exon 11	c.1719C>G	p.Ser573Arg	PM2, PP2, PP3	VOUS	Seizure with mild fever at 4.5 y of age.	Novel
15 y	M	Exon 11	c.1719C>G	p.Ser573Arg	PM2, PP2, PP3	VOUS	Afebrile seizures, Attention deficit.	Novel
35 y	M	Exon 11	c.1719C>G	p.Ser573Arg	PM2, PP2, PP3	VOUS	Seizure	Novel
13 y	M	Exon 13	c.1423_1424 delinsCT	p.Ala475Leu	PM2, PP2, PP3	VOUS	Seizures in sleep, blank stares, generalized tonic-clonic seizure, uplifting of eyes,	Novel
13 y	M	Exon 13	c.1423_1424 delinsCT	p.Ala475Leu	PM2, PP2, PP3	VOUS	Epilepsy,	Novel
Unknown	F	Exon 28	c.4750T>C	p.Phe1584Leu	PM2, PP2, PP3	VOUS	Focal and generalized tonic clonic seizures	Novel
7 y	F	Exon 4	c.136G>A	p.Glu46Lys	PM1, PM2, PP2, PP3	VOUS	Seizures	Novel

M: male, F: female. [a]*All were novel mutations except for this child.*