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Lack of meaningful genotype-phenotype association in *SCN1A*-related infantile-onset epileptic encephalopathies

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Abstract

Background & Objective: SCN1A gene which encodes for sodium channel alpha 1 subunit has been found to be the most common mutated gene in patients with epilepsy. This study aims to characterize the *SCN1A* mutations as well as to describe genotype and phenotype association in children with *SCN1A*-related infantile-onset epileptic encephalopathies in Malaysia. *Methods:* Children with infantile-onset epileptic encephalopathy mostly suspected to have Dravet syndrome who had mutational analysis for *SCN1A* gene from hospitals all over Malaysia were included in the study. Their epilepsy syndrome diagnosis was classified into severe myoclonic epilepsy in infancy and its variants. Polymerase chain reaction and bidirectional sequencing were used to identify *SCN1A* mutations. *Results:* A total of 38 children with heterozygous mutations were analysed, 22 (57.9%) of which were novel mutations. Truncated mutations were the most common mutation type (19, 50%). Other mutation types were missense mutations (14, 36.8%), splice site mutations (4, 10.5%) and in-frame deletion (1, 2.6%). The mean age of seizure onset was 4.7 months. Seizure following vaccination was observed in 26.3% of the children. All of them had drug resistant epilepsy. There was no significant association between the type of mutation with the syndromic diagnosis, age of seizure onset, tendency of the seizures to cluster or having status epilepticus, mean age when developmental delay was observed and response to various antiepileptic drugs.
Conclusion: This study expands the spectrum of *SCN1A* mutations and proves the importance of *SCN1A* gene testing in diagnosing infantile-onset epileptic encephalopathies patients. Although, our study does not support any clinically meaningful genotype-phenotype association for *SCN1A*-related infantile-onset epileptic encephalopathies, the clinical characteristics of our cohort are similar to those that have been described in previous studies.

Key words: SCN1A mutations, epileptic encephalopathies, genotype-phenotype

INTRODUCTION

Epilepsy that has its onset during infancy is generally difficult to control and often leads to poor long term development and intellectual outcome. Many of them are now categorized as epileptic encephalopathies which are characterized by frequent severe seizures, and/or prominent interictal epileptiform discharges on the electroencephalogram, developmental delay or deterioration, and usually a poor prognosis. The epileptiform abnormalities themselves are believed to contribute to the progressive disturbance in cerebral function.[1] They include Ohtahara syndrome, early myoclonic

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epileptic encephalopathy, West syndrome, severe myoclonic epilepsy in infancy (SMEI or Dravet syndrome), and other related epilepsy syndromes. A number of gene mutations have been linked to these infantile-onset epileptic encephalopathies such as Aristaless-related homeobox (*ARX*), Cyclin-dependent kinase-like 5 (*CDKL5*), syntaxin-binding protein 1 (*STXBP1*), solute carrier family 25 member 22 (*SLC25A22*), non erythrocytic a-spectrin-1 (*SPAN1*), phospholipase Cb1 (*PLCb1*), membrane associated guanylate kinase inverted-2 (*MAGI2*), polynucleotide kinase 30-phosphatase (*PNKP*), protocadherin 19 (*PCDH19*), pyridoxamine 5-primephosphate oxidase (*PNPO*) and sodium channel neuronal type 1a subunit (*SCN1A*).[2]

SCN1A gene is the most commonly mutated gene in many types of epilepsy, known as 'super culprit' gene. *SCN1A* gene which maps on chromosome 2q24.3 consists of 26 exons and is expressed in the central and peripheral nervous system and in cardiac myocytes. The sodium channel protein consists of a highly processed ~260kDa α subunit that comprises four homologous domains termed I-IV each with six transmembrane segments (S1-S6). Mutations in *SCN1A* occur in approximately 80% of patients with SMEI (Dravet syndrome).[3-5] Besides that, *SCN1A* mutations are also found in other forms of infantile-onset epileptic encephalopathies such as SMEI-borderland (SMEB), cryptogenic generalized epilepsies, cryptogenic focal epilepsies[6], migrating partial seizures of infancy (MPSI)[7-8] and hemiconvulsion-hemiplegia-epilepsy syndrome (HHES).[9] To date over 700 mutations have been reported in public version of Human Genome Mutation Database (HGMD) which can be access at http://www.hgmd.org. *SCN1A* mutations in SMEI are located throughout the gene, with 50% of them are truncating mutation and the remaining 50% comprising of missense, splice site and deletion mutation.[4] About 95% of *SCN1A* mutations arise *de novo* while the remaining cases are familial mutations with milder phenotypes in other family members often consistent with genetic epilepsy with febrile seizures plus (GEFS+) spectrum.[6]

Currently in Malaysia, infantile-onset epileptic encephalopathies are diagnosed largely by the clinical manifestations and progression of the disease and most of them are diagnosed late. The objective of this study is to describe the clinical and mutational characteristics of Malaysian children with *SCN1A*-related infantile-onset epileptic encephalopathies and to determine if there is any clinically important genotype-phenotype correlation.

METHODS

Patients and clinical analysis

Children aged less than 18 years old with infantile onset epileptic encephalopathies mostly suspected to have Dravet syndrome who had mutational analysis for *SCN1A* gene from six regional hospitals with paediatric neurologists in Malaysia were included in the study from April 2014 to May 2015. We only included those who had their seizure onset at less than two years old. Their epilepsy phenotypes were classified by same paediatric neurologist (TB Khoo) who was masked to the mutation type to maintain diagnostic consistency as SMEI(Dravet syndrome), SMEB(SMEI borderland) and other related epilepsy syndromes as described previously by Harkin *et al*.[6] SMEB was further divided into SMEB-M (no myoclonic seizures), SMEB-SW (no generalized spike-wave activity on EEG), SMEB-O (has more than one atypical features) and ICEGTC (same course as of SMEI but only has generalized tonic-clonic seizures). We excluded those infantile-onset epileptic encephalopathies not associated with *SCN1A* mutation such as Ohtahara syndrome, early myoclonic encephalopathy, West syndrome and epileptic encephalopathies due to known metabolic, structural, chromosomal abnormality or other genetic mutations. Their electroclinical data were compiled with a standard study proforma which included detail seizure characteristics, developmental progression, electroencephalographic (EEG) and neuroimaging findings as well as their response to antiepileptic drug (AED) treatment.

Direct sequencing of SCNIA gene

Peripheral blood samples were taken from patients after informed consent obtained from their parents. Genomic DNAs were extracted from EDTA-treated whole blood samples using QiaAmp DNA Blood kit (Qiagen, Germany). Concentration and purity of DNA were measured using Nanodrop Spectrophotometer. Polymerase chain reaction (PCR) was carried out using 30 set of primers to amplify 26 exons and flanking intron of *SCN1A* gene. PCR products were then cycle sequenced using Big Dye Terminator v3.1 chemistry, (Applied Biosystem, CA, USA) purified and analyzed on Genetic Analyzer ABI 3500 (Applied Biosystem, CA, USA). Sequencing results were then analysed

for mutation using SeqScape software v3.0. Nucleotide sequences were compared to the reference sequence NM_00116596.3 to identify sequence changes. The significance of novel mutations was evaluated by allele frequencies in 100 normal alleles and pathogenicity prediction analysis using MutationTaster2 program (http://www.mutationtaster.org).

Multiple ligation probe amplification (MLPA)

MLPA were conducted to the samples that have no *SCN1A* mutations detected by bidirectional sequencing. MLPA analysis performed using SALSA MLPA kit P137-B2 *SCN1A* (MRC Holland, The Netherlands) according to the manufacturer instructions. About 50ng of DNA for all samples were used for ligation and amplification procedures of MLPA using Thermocycler Pro S (Eppendorf, Germany). All amplified fragments were separated using capillary electrophoresis on Genetic Analyzer 3500 (Applied Biosystem, CA, USA). Data were analysed using Gene Markerv1.85 software (Soft-Genetics, USA). The reference range for normal copy number was set at 0.75-1.3.

Ethical approval was obtained from the Medical and Research Ethics Committee, Ministry of Health, Malaysia. (NMRR ID: NMRR-13-181-15030)

Statistical analysis

Any mutation detected was compared to the existing mutation database such as *SCN1A* Variant Database and HGMD. The mutations found were categorized as truncation (nonsense or frameshift), missense, splice site and deletion. Genotype-phenotype correlations were carried out using independent-sample t-test or Mann-Whitney U test for continuous data with normal and non-normal distribution respectively and Fisher's Exact test for categorical data using SPSS software, version 20 to illustrate the relationship between the type of mutations with the phenotypic expression such as age of seizure onset, clinical seizure semiology, syndromic diagnosis, age of developmental delay or regression, developmental outcome and response to various anti-epileptic drugs treatment. Patients with splice site mutation and deletion were excluded from the genotype-phenotype correlations analysis because of their small sample size. Differences were considered significant when p was < 0.05.

RESULTS

Clinical characteristics

One hundred and forty seven children with infantile-onset epileptic encephalopathies were tested and 54 patients (36.7%) were found to have heterozygous mutations of *SCN1A* gene. Complete clinical information was only available for 38 patients. Among them, 2 had SMEI, 18 had SMEB-SW, 1 had SMEB-M, 16 had SMEB-O and 1 had ICEGTC.

Their clinical characteristics are shown in Table 1. The mean age of seizure onset was 4.7 months (SD$\pm$1.9 months). Thirty-three (86.8%) had fever provoked seizures and 10 of them (26.3%) had seizure following vaccination. Eighty one percent of them had status epilepticus and feature of seizure clustering. All of them had normal developmental milestone initially and the mean age when developmental delay or regression noted was 22.6 months. At a mean follow up age of 93 months (range: 14-246 months), all of them except one had learning disabilities (mild in 8, moderate in 20, severe / profound in 8). Other comorbidities include autism (19, 50%), attention deficit hyperactivity disorder (14, 36.8%), ataxia (13, 34.2%), spasticity (5, 13.1%) and dyskinesia (5, 13.1%).

All of them had seizures that are drug resistant. Their longest seizure-free period ranged from less than 1 month to 24 months with a mean of 3.5 months despite being on various combinations of AEDs. Sodium valproate, clobazam, topiramate, levetiracetam and stiripentol were found to be helpful AEDs. Carbamazepine and lamotrigine worsened seizures in those who were tried on them. Benzodiazepine (either rectal diazepam or buccal midazolam) were found to be more beneficial during clustering of seizures or status epilepticus in 57.9% of the patients compared to phenytoin or phenobarbitone in 28.9% and 26.3% respectively.

SCN1A mutational analysis

Truncation mutation was the most common mutation detected including nonsense mutations (31.6%, 12/38) and frameshift mutations (18.4%, 7/38). Missense mutations accounted for 36.8% (14/38). The remaining were splice site mutations exhibited in 4 patients (10.5%) and one had microdeletion (2.6%). Frameshift mutations were comprised of one duplication (c.3001dupG), one insertion (c.2068_2069instT) and 5 microdeletions (c.5788delC, c.3099delT, c.654_655delCA,

Table 1: Clinical, EEG and MRI feature of 38 *SCN1A*-related infantile-onset epileptic encephalopathies patients.

Pt no.	Age of onset	Phenotype	Seizure types	Seizure after vaccination	Status epilepticus	Tendency for seizures to cluster	Age developmental delay noted	Current developmental status	Other Co-morbidities	Interictal EEG	MRI brain	Family history
Truncation Mutations												
1	2 mo	SMEI	FS, HCFS, MS, GTCS, AA	N	Y	N	12 mo	Severe LD	Autistic	GSW/PSW	Normal	Brother had SMEI, Grandmother had FS
2	2 mo	SMEB-SW	FS, HCFS, MS, GTCS, CPS	N	Y	Y	12 mo	Severe LD	Ataxia, ADHD	Normal	Normal (CT brain)	Nil
3	3 mo	SMEB-SW	FS, HCFS, MS, GTCS, CPS	Y	Y	N	21 mo	Mild LD	ADHD, Ataxia	Normal	Normal	Nil
4	5 mo	SMEB-SW	FS, HCFS, MS, GTCS, CPS	N	Y	NA	24 mo	Moderate LD	Speech regression	MF	Normal	Nil
5	5 mo	SMEB-SW	FS, HCFS, MS, GTCS	N	Y	Y	24 mo	Moderate LD	ADHD	Normal	Normal (CT brain)	Uncle and granduncle had FS
6	5 mo	SMEB-SW	FS, HCFS, MS, GTCS, CPS	N	Y	Y	14 mo	Mild LD	Autistic, ADHD Ataxia	Normal	Normal	Nil
7	6 mo	SMEB-SW	FS, HCFS, MS, GTCS, CPS	N	Y	Y	NA	Moderate LD	Autistic, ADHD	MF	Normal	Mother had FS
8	6 mo	SMEB-SW	FS, MS, GTCS	N	Y	Y	20 mo	Moderate LD	Ataxia, Spasticity	FD	Normal	Nil
9	6 mo	SMEB-SW	FS, HCFS, MS, GTCS	N	Y	NA	24 mo	Mild LD	Nil	Normal	Normal	Father had FS
10	8 mo	SMEB-SW	FS, HCFS, MS, GTCS, CPS	Y	Y	Y	36 mo	Moderate LD	Autistic	Normal	Normal	Granduncle had epilepsy

Pt no.	Age of onset	Phenotype	Seizure types	Seizure after vaccination	Status epilepticus	Tendency for seizures to cluster	Age developmental delay noted	Current developmental status	Other Co-morbidities	Interictal EEG	MRI brain	Family history
11	3 mo	SMEB-O	FS, HCFS, GTCS, CPS	N	Y	NA	18 mo	Moderate LD	Autistic, Ataxia	FD	Left cerebral atrophy	Nil
12	3 mo	SMEB-O	FS, HCFS, GTCS, CPS	N	Y	NA	24 mo	Moderate LD	Nil	Normal	Normal	Nil
13	3 mo	SMEB-O	FS, HCFS, GTCS, CPS	N	Y	N	15 mo	Moderate LD	Nil	Normal	Normal	Nil
14	4 mo	SMEB-O	FS, HCFS, GTCS	Y	Y	Y	48 mo	Mild LD	ADHD	Normal	Normal	Nil
15	4 mo	SMEB-O	FS, HCFS, GTCS	N	Y	Y	12 mo	Profound LD	Dyskinesia	Slow background	Normal	Two brothers had epilepsy
16	5 mo	SMEB-O	FS, HCFS, GTCS, CPS	N	Y	Y	NA	Moderate LD	Autistic, Ataxia	FD	Normal	Nil
17	6 mo	SMEB-O	FS, HCFS, GTCS, TS	N	N	Y	12 mo	Moderate LD	Autistic	Normal	Normal	Nil
18	8 mo	SMEB-O	FS, HCFS, GTCS, CPS	N	N	Y	36 mo	Moderate LD	Autistic, ADHD	MF	Non-specific changes	Sister had SMEB
19	5 mo	ICEGTC	FS, GTCS	Y	Y	Y	60 mo	Moderate LD	Dyskinesia	FD	Normal	Sister and aunt had FS
Missense Mutations												
20	3 mo	SMEI	FS, HCFS, MS, CPS, GTCS	Y	Y	NA	24 mo	Mild LD	ADHD	GSW/PSW/ MF	Normal	Uncle has epilepsy
21	3 mo	SMEB-SW	FS, HCFS, MS, GTCS, AA	N	Y	Y	34 mo	Moderate LD	Autistic, ADHD Ataxia, Dyskinesia	FD	Normal	Cousin had epilepsy
22	3 mo	SMEB-SW	FS, MS, GTCS, CPS, AA	Y	Y	Y	12 mo	Moderate LD	Autistic	FD	Normal	Grandaunt had epilepsy

Pt no.	Age of onset	Phenotype	Seizure types	Seizure after vaccination	Status epilepticus	Tendency for seizures to cluster	Age developmental delay noted	Current developmental status	Other Co-morbidities	Interictal EEG	MRI brain	Family history
23	3 mo	SMEB-SW	FS, HCFS, MS, GTCS	N	Y	Y	24 mo	NA	Autistic, Hypotonia, Dyskinesia	MF	Cerebral atrophy	NA
24	4 mo	SMEB-SW	FS, HCFS, MS, GTCS, CPS	N	Y	Y	13 mo	Moderate LD	Autistic, ADHD, Ataxia	Normal	Cerebral atrophy	Nil
25	4 mo	SMEB-SW	FS, HCFC, MS, GTCS, CPS	N	Y	N	NA	Normal	Paroxysmal ataxia	Normal	Not done	Nil
26	6 mo	SMEB-SW	FS, MS, GTCS, CPS, AA	N	Y	Y	9 mo	Profound LD	Spasticity	MF	Normal	Nil
27	3 mo	SMEB-M	FS, HCFS GTCS	N	Y	NA	20 mo	Mild LD	Ataxia	GSW/PSW	Normal (CT brain)	Nil
28	1 mo	SMEB-O	HCFS, GTCS, CPS	Y	Y	N	11 mo	Severe LD	Autistic, Spasticity	Normal	Cerebral atrophy	Nil
29	4 mo	SMEB-O	FS,HCFS, GTCS, CPS, AA	Y	Y	Y	12 mo	Moderate LD	Ataxia	FD	Right temp. lobe atrophy	Aunt had FS, Uncle had epilepsy
30	4 mo	SMEB-O	FS, GTCS, CPS	N	Y	Y	24 mo	Profound LD	Autistic, Ataxia, Spasticity	FD	Normal	Nil
31	5 mo	SMEB-O	HCFS, CPS, AA	N	Y	Y	24 mo	Moderate LD	ADHD	GSW/PSW/ MF	Normal	Nil
32	6 mo	SMEB-O	FS, HCFS, MS, CPS	Y	N	Y	18 mo	Mild LD	Autistic, Ataxia	Normal	Normal	Nil
33	7 mo	SMEB-O	HCFS, GTCS, CPS	N	Y	Y	36 mo	Severe LD	Autistics, ADHD	FD	Normal	Nil

Pt no.	Age of onset	Phenotype	Seizure types	Seizure after vaccination	Status epilepticus	Tendency for seizures to cluster	Age developmental delay noted	Current developmental status	Other Co-morbidities	Interictal EEG	MRI brain	Family history
Splice site Mutations												
34	3 mo	SMEB-SW	HCFS, MS, GTCS, CPS, AA	N	Y	Y	21 mo	Severe LD	Autistic, Spasticity, Dyskinesia	Normal	Normal	Brother had epilepsy and sudden death
35	6 mo	SMEB-SW	FS, MS, GTCS, AA	N	N	Y	24 mo	Moderate LD	Nil	MF	Normal	Brother and aunt had FS
36	7 mo	SMEB-SW	FS, HCFS, MS, GTCS, AA	Y	N	Y	34 mo	Moderate LD	Autistic, ADHD	MF	Normal	Nil
37	9 mo	SMEB-O	FS, GTCS, CPS	N	N	N	16 mo	Mild LD	Autistic	Normal	Normal	Nil
Microdeletion												
38	8 mo	SMEB-O	HCFS, GTCS, CPS	N	N	Y	24 mo	Moderate LD	Autistic	FD	Normal	Two granduncles had epilepsy

FS: Febrile seizure, HCFS: Hemiclonic or focal seizures, MS: Myoclonic seizures, GTCS: Generalised tonic-clonic seizure, CPS: Complex partial seizures, AA: Atypical absences, TS: Tonic seizures N: No, Y: Yes, NA: Information not available, LD: Learning disability, ADHD: Attention deficit hyperactivity disorder, GSW: Generalised spike wave discharges, PSW: Polyspike wave discharges, MF: Multifocal discharges, FD: Focal Discharges

Table 2: *SCN1A* mutations detected in 38 infantile-onset epileptic encephalopathies patients.

Pt No	Phenotype	cDNA	Protein	Exon	Subunit location	Reported
Truncation mutations						
1	SMEI	c.4906C>T	p.Arg1636*	26	DIV-S4	No
2	SMEB-SW	c.4547C>A	p.Ser1516*	24	DIII-DIV	Sugawara, 2002
3	SMEB-SW	c. 5788delC	p.Leu1930PheFs*2	26	C-terminal	No
4	SMEB-SW	c.5656C>T	p.Arg1886*	26	C-terminal	Mancardi,2006
5	SMEB-SW	c.3099delT	p.Phe1033LeuFs*13	16	DII-DIII	No
6	SMEB-SW	c.3943_3949delCTCAGGA	p.Leu1315HisFs*2	20	DIII-S4	No
7	SMEB-SW	c.1702C>T	p.Arg568*	11	DI-DII	Ohmori, 2002
8	SMEB-SW	c.2134C>T	p.Arg712*	12	DI-DII	Sugawara, 2002
9	SMEB-SW	c.1152G>A	p.Trp384*	8	DI S5-S6	Harkin, 2007
10	SMEB-SW	c.5155 C>T	p.Gln1719*	26	DIV S5-S6	No
11	SMEB-O	c.942G>A	p.Trp314*	6	DI S5-S6	No
12	SMEB-O	c.5734C>T	p.Arg1912*	26	C-terminal	Fukuma, 2004
13	SMEB-O	c.506C>G	p.Ser169*	4	D1-S2	No
14	SMEB-O	c.2068_2069instT	p.Arg690MetFs*39	12	DI-DII	No
15	SMEB-O	c.3079A>T	p.Lys1027*	16	DII-DIII	Ohmori (2002)
16	SMEB-O	c.1813_1814delAG	p.Arg605ArgFs*21	11	DI-DII	No
17	SMEB-O	c.654_655del CA	p.Phe218LeuFs*58	5	DI-S4	No
18	SMEB-O	c.3829C>T	p.Gln1277*	19	DIII S2-S3	Hattori (2008)
19	ICEGTC	c.3001dupG	p.Ala1001GlyFs*4	16	DII-DIII	No
Missense mutations						
20	SMEI	c.247T>G	p.Tyr83Asp	1	N-terminal	No
21	SMEB-SW	c.5129T>G	p.Phe1710Cys	26	DIV S5-S6	No
22	SMEB-SW	c.1177C>T	p.Arg393Cys	9	DI S5-S6	Mancardi, 2005
23	SMEB-SW	c.4649T>G	p.Leu1550Arg	25	DIV-S1	No
24	SMEB-SW	c.838T>C	p.Trp280Arg	6	DI S5-S6	Nabbout, 2003
25	SMEB-SW	c.2836C>T	p.Arg946Cys	15	DII S5-S6	Fukuma (2004)
26	SMEB-SW	c.280A>C	p.Asn94His	2	N-terminal	No
27	SMEB-M	c.773T >C	p.Leu258Pro	6	DI-S5	No
28	SMEB-O	c.1034G>A	p.Cys345Tyr	8	DI S5-S6	No
29	SMEB-O	c.5345T>C	p.Ile1782Thr	26	DIV-S6	No
39	SMEB-O	c.424T>C	p.Cys142Arg	3	DIS1	No
31	SMEB-O	c.2837G>A	p.Arg946His	15	DII S5-S6	Fukuma, 2004
32	SMEB-O	c.2837G>A	p.Arg946His	15	DII S5-S6	Fukuma, 2004
33	SMEB-O	c. 4072T>C	p.Trp1358Arg	21	DIIIS5	No
Splice site mutations						
34	SMEB-SW	c.2589+3A>T	Splice site	IVS 14		Harkin, 2007
35	SMEB-SW	c.1377+1G>A	Splice site	IVS 9		Depienne, 2009
36	SMEB-SW	c.2415+2T>A	Splice site	IVS 13		No
37	SMEB-O	c.602+1G>A	Splice site	IVS 4		Fujiwara,2003
Microdeletion						
38	SMEB-O	c.4786_4788delCGC	p.Arg1596del	25	DIV S2-S3	No

c.1813_1814delAG, c.3943_3949delCTCAGGA) leading to premature stop codon which were predicted to produce non-functional protein. The detailed *SCN1A* mutations were shown in Table 2. Of the 38 mutations identified, 22 mutations have not been previously reported. None of the novel mutations were present in our 100 normal alleles excluding the probability of polymorphism. MutationTaster2 predicted all novel mutations to be disease causing mutation.

Further investigation using MLPA on the 93 samples that have no *SCN1A* point mutations showed neither large deletion nor duplication, indicating that this type of mutation was not common. A nucleotide change at c.2837G>A (p.Arg946His) was detected in 2 unrelated patients (Patient 31 & 32) suggesting a recurrent mutation. No mutation was detected in parents of Patient 9, 15 and 19, however we could not confirm whether the mutation has arisen *de novo* or germinal mosaicism as no analysis been carried out on their sibling. On the other hand, Patient 1 and 18 shared the same mutation with their sibling but not present in their parents, thus suggesting germinal mosaicism.

Genotype-phenotype correlation

There is no significant association between the type of mutation and the syndromic diagnosis, age of seizure onset, likelihood of seizures after vaccination, tendency of the seizures to cluster or having status epilepticus, age when developmental delay or regression was observed as shown in Table 3 and response to various AEDs as shown in Table 4.

Table 3: Genotype-Phenotype Correlations of the 38 patients

Clinical characteristics	Truncation (n=19)	Missense (n=14)	Splice site (n=4)	Deletion (n=1)	All (n=38)
Syndromic Diagnosis*					
– SMEI	1	1	0	0	2
– SMEB-SW	9	6	3	0	18
– SMEB-M	0	1	0	0	1
– SMEB-O	8	6	1	1	16
– IGEGTC	1	0	0	0	1
Age of seizure onset+ (Mean / Median in months)	4.7/ 5	4.0/4	6.25/ 6.5	8/8	4.7/4.5
Seizure after vaccination*	(4/19)21.1%	(5/14)35.7%	(1/4)25%	0	26.3%
Status epilepticus*	(17/19)89.5%	(13/14)92.9%	(1/4)25%	0	81.6%
Tendency for seizures to* cluster	(12/15)80%	(10/12)83.3%	(3/4)75%	(1/1)100%	81.2%
Longest seizure-free period# (Mean / Median in months)	3.2/3	4.5/2	4 / 3	1 / 1	3.7 / 2.5
Age when developmental delay was noted# (Mean / Median in months)	24.2/21	19.8/20	26.3/22.5	24/24	22.6/21
Family history of febrile seizures / epilepsy*	(8/19)42.1%	(4/13)30.8%	(2/4)50%	(1/1)100%	40.5%

+Not significant *(p* value derived using Independent samples t-test comparing truncation and missense mutation only)
Not significant *(p* value derived using Independent samples Mann-Whitney U test comparing truncation and missense mutation only)
* Not significant (*p* value derived using Fisher's Exact test comparing truncation and missense mutations only)

SMEI: Severe myoclonic epilepsy in infancy / Dravet syndrome,
SMEB-SW: SMEI borderland with no generalized spike-wave activity on EEG
SMEB-M: SMEI borderland with no myoclonic seizures,
SMEB-O: SMEI borderland with more than one atypical feature
ICEGTC: SMEI borderline with same course as SMEI but only has generalized tonic-clonic seizures

Table 4: Response* to anti-epileptic drugs according to the type of mutations.

Mutation	Truncation (n=19)		Missense (n=14)		Splice site (n=4)		Deletion (n=1)		All (n=38)	
seizures / AED	↓	↑	↓	↑	↓	↑	↓	↑	↓	↑
Sodium valproate	8	1	7	0	1	0	0	0	16	1
Clobazam	8	0	6	0	2	1	0	0	16	1
Topiramate	5	1	5	0	3	0	0	0	13	1
Levetiracetam	2	1	4	1	1	1	1	0	8	3
Stiripentol	2	0	1	0	1	0	0	0	4	0
Carbamazepine	0	4	0	3	0	1	0	1	0	9
Lamotrigine	1	4	0	4	0	3	0	0	1	11

* Not all patients received all the antiepileptic drugs as listed, ↓: reduce, ↑: worsen

DISCUSSION

The commonest types of *SCN1A* mutations among Malaysian children are the truncating mutations (50%), followed by missense mutations (36.8%), splice site mutations (10.5%) and small deletion (2.6%). Meng *et al*. reported 81.8% of SCN1A mutations as novel.[10] However, it is slightly lower in our study (57.9%). Truncation mutations were spread throughout the gene whereas most of the missense mutations were localized at the transmembrane region of the protein, particularly in the S5-S6 region that functions as ion pore channel as illustrated in Figure 1. These appear to be consistent with study by Zuberi *et al.*[11]

Patient 1 harbours changes from C to T at position 4906 which was predicted to produce truncated protein at codon 1636 (p.Arg1636*). Sequencing analysis of family samples showed that the same mutation was also detected in the younger brother who was diagnosed with SMEI whereas no mutation detected in parent's sample. Patient 18 exhibit changes c.3829C>T (p.Gln1277*) that was also found in his symptomatic sister but not present in their parents as shown in Figure 2.

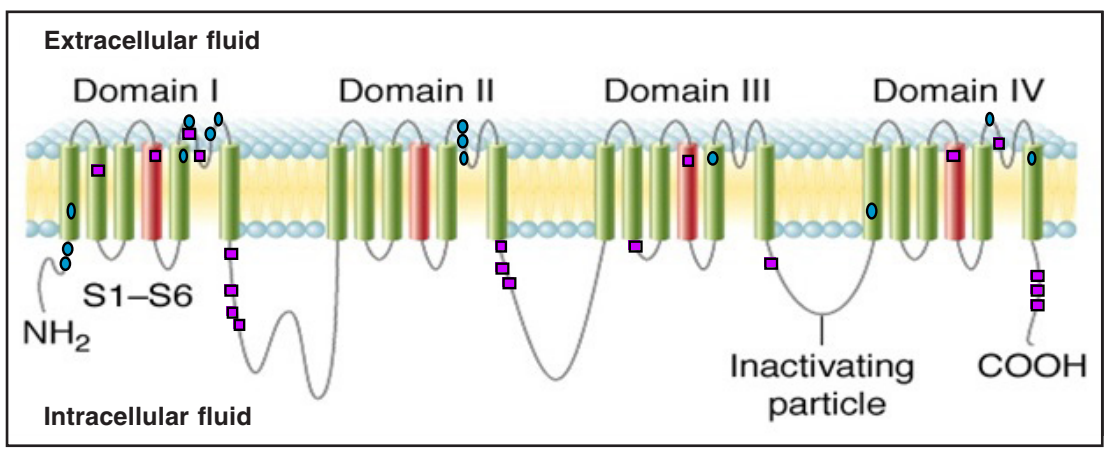

Figure 1: Schematic representation of the mutations identified in this study on the sodium channel alpha 1 subunit protein. These mutations were spread throughout the gene with the majority of missense mutation were localized at the transmembrane regions of the protein (12/14) in particular the S5-S6 domain that functions as ion pore channel. In contrast most of truncation mutations (12/19) were positioned at the intracellular loops of the protein.

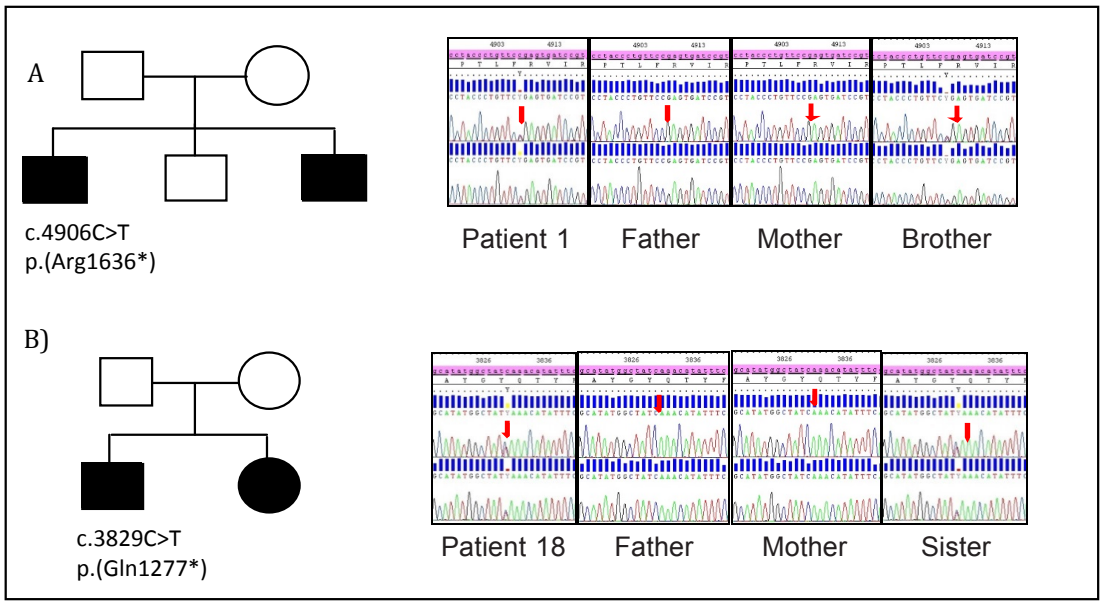

Figure 2: Family study and sequencing electropherogram of two patients with possibility of germinal mosaicism. A) c.4906C>T mutation in Patient 1. The same mutation was also present in symptomatic brother. B) c.3829C>T mutation in Patient 18 and his younger sister.

The absence of mutation in parental samples of Patient 1 and Patient 18 suggested the occurrence of germinal mosaicism. Germinal mosaicism is defined as a state in which some of the germ cells of the gonad are of a form not present in either parent, because mutation is an intermediate progenitor of these cells.[12] Germinal mosaicism in *SCN1A* gene has been reported in three different cases.[12-14] Identification of germline mosaicism is crucial as it will increase the risk of subsequent affected offspring as well as for genetic counselling.

It is of note that only 2 out of 38 of our cohort had SMEI (Patient 1, 20) and the rest had SMEB phenotypes. This could be due to the shorter duration of follow up in some of the patients before the *SCN1A* gene mutation test was requested as well as the stricter diagnostic criteria used in this study as described by Harkin *et al*.[6] However, the clinical characteristics of our cohort are similar to those that have been described in previous studies.[15-17]

Our study also showed that phenotypic subdivisions of SMEI (or Dravet syndrome) and its variants such as SMEB-SW, SMEB-M, SMEB-O and ICEGTC were unhelpful as it does not correlate to the type of mutations, the patients' developmental outcome or response to AED treatment. This study also supports Guerrini's proposal that Dravet syndrome be designated as a syndrome spectrum that also embraces SMEB and for those that exhibit a less severe or incomplete form of the syndrome be defined as 'mild form' of Dravet syndrome.[18] In young infants with recurrent seizures that are often prolonged and precipitated by fever when molecular genetic testing for *SCN1A* mutation is positive and the clinical picture still unclear, a more appropriate term is *SCN1A* gene-related epilepsy.[18]

Our study does not support any clinically meaningful genotype-phenotype association for *SCN1A*-related infantile-onset epileptic encephalopathies. This was also observed in previous studies[17,19] and patients with same mutation could have different phenotypic expression (Patients 31 and 32). Although similar to study by Nabbout *et al*.[20], 89.5% of our patients with truncating mutation had focal or hemiclonic seizures, however, it was also present in 78.6% and 50% of our patient with missense and slice site mutations respectively. In contrast, study by Zuberi *et al*. with a larger cohort noted there was no difference between the presence of different seizure types between those with truncating and missense mutation.[11]

In the study by Zuberi *et al*, the mean age at onset of seizure was earlier in those with truncating compared to missense mutation for prolonged seizures, myoclonic seizures and atypical absence seizures.[11] However, these data are often not

available at the early phase when an infant is suspected to have *SCN1A*-related epileptic encephalopathy and the clinical relevance of the types of mutation is questionable. In fact, we did not detect any significant difference between truncating and missense mutations on our cohort with regards to their mean age of seizure onset, rate of seizure after vaccination, status epilepticus, tendency for seizures to cluster, mean age when developmental delay was noted and family history of febrile seizures or epilepsy.

Similarly, we also did not find any significant difference in the response to treatment between truncating and missense mutation in our cohort though their numbers are too few to make a definite conclusion. However, AEDs that are found to be most helpful are sodium valproate, clobazam, topiramate, stiripentol and to a lesser extent, levetiracetam. Carbamazepine and lamotrigine are found as in previous studies to worsen seizures.[21-23]

There are some important limitations in this study because the clinical information was obtained retrospectively through review of the case records, smaller sample size compared to previous study[11], and few of our younger patients may have not yet manifested their full clinical features. It is also important to note that this study included patients with infantile-onset epileptic encephalopathies only and could not be generalized to those with genetic epilepsy with febrile seizure plus spectrum due to *SCNIA* gene mutation.

In conclusion, our study does not support any clinically meaningful genotype-phenotype association for *SCN1A*-related infantile-onset epileptic encephalopathies. However, confirming the diagnosis with *SCN1A* mutational analysis will remain essential especially for the younger patients as it could prevent additional investigations, alter treatment approach, influence medication choice, improve seizure control and assist in accessing additional therapies.[24]

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DISCLOSURE

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