Original Article

Sodium channels of *SCN1A* gene mutations in generalized epilepsy with febrile seizure plus (GEFS+) spectrum related to autism

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Abstract

Background Mutations in the α–subunit of the first neuronal sodium channel gene *SCN1A* have been demonstrated for generalized epilepsy with febrile seizures plus (GEFS+), severe myoclonic epilepsy in infancy (SMEI), and borderline SMEI (SMEB). *SCN1A* mutations are also described in patients with psychiatric disorders such as autism.

Objective To identify the mutations of *SCN1A* gene in patients with GEFS+ spectrum which may be related to autism.

Methods We examined four patients with autism and GEFS+ spectrum who were admitted to the Department of Child Health, Sardjito Hospital, Yogyakarta, Indonesia. Diagnosis of autism was based on DSM-IV/ICD X criteria. Mutations in *SCN1A* were identified by PCR amplification and denaturing high-performance liquid chromatography analysis, with subsequent sequencing.

Results There were four patients, all boys, aged 1.8 year to 7 years. The phenotypes of epilepsy were GEFS+ in one patient, SMEB in one patient and SMEI in two patients. Sequencing analysis revealed a G-to-A heterozygous transition which was detected at nucleotide c.4834G>A (p.V1612I) in exon 25. Other single nucleotid polymorphisms (SNPs) were c.383+66T>C in intron 2, c.603-91G>A and c.603-106G>T in intron 4, c.965-21C>T in intron 6, c.1028+21T>C in intron 7, c.2173G>A in exon 12 and c. 2177-38C>A, c.2177-12delT, c.2176+44C>T in intron 12.

Conclusion In this study, we reported the first cases with mutation in *SCN1A* gene in GEFS+ spectrum related to autistic patients in Indonesian population, which showed a missense mutation p.V1612I. **[Paediatr Indones. 2010;50:125-32]**.

Keywords: mutation, SCN1A, Generalized epilepsy with febrile seizures plus; Severe myoclonic epilepsy in infancy; autistic spectrum disorder

Mutations in the α –subunit of the first neuronal sodium channel gene *SCN1A* have been demonstrated for generalized epilepsy with febrile seizures plus (GEFS+),[1-3] severe myoclonic epilepsy in infancy (SMEI), and borderline SMEI (SMEB).[4-6] Moreover, *SCN1A* mutations were also described in patients with psychiatric disorders such as autism, Asperger syndrome and panic disorder with seizures.[7,8]

Patients with GEFS+ (MIM 604233) are characterized by febrile seizures that persist beyond age six years and by heterogeneous afebrile seizures that may include tonic-clonic, atonic, myoclonic and absence seizures.[9] However, the last terminology of GEFS+ spectrum according to Scheffer and

Berkovic was the spectrum illustrated the phenotypic heterogeneity seen in GEFS+ families ranging from benign phenotypes such as FS to severe epileptic encephalopathies such as SMEI.10 Meanwhile, SMEI (OMIM 182389) is a rare disorder, characterized by various types of generalized and partial seizures, including myoclonic seizures. Seizures usually appear during the first year of life and are initially induced by fever. Electroencephalography (EEG) recordings reveal generalized spike-wave and polyspike-wave, early photosensitivity and focal abnormalities. Psychomotor development is retarded from the second year of life along with ataxia and refractoriness to drug therapy.[11] On the otherhand, SMEB is a diagnosis in patients who do not fulfill all the SMEI criteria.

Autism (MIM 209850) is a complex psychiatric disorder characterized by impaired communication and social skills, restricted and repetitive behaviours.[12] Autism is found in 13 per 10,000 population, and nowadays there is a perception that the incidence of autism is increasing. Definitely, there is heightened awareness and concern about the prevalence of this disorder. However, no evidence that this is a true increase rather than an increase in awareness of this situation.[13,14] Autism is not a disease but a syndrome with multiple non-genetic and genetic causes. One-third of autistic patients experience seizures.[7] A susceptibility locus for autism was mapped near a cluster of voltage-gated sodium channel genes on chromosome 2. To know these *SCN1A* mutation as a candidate for the autism susceptibility locus, we screened for four patients who suffered from GEFS+ spectrum and autistic spectrum disorder.

Methods

Criteria diagnosis for GEFS+ and SMEI were done according to International League Against Epilepsy (ILAE) criteria[15] and to the nomenclatur introduced by Scheffer and Berkovic.[9] Autism diagnosis was based on the accepted criteria of DSM-IV/ICD-10.[16,17]

Molecular analysis was carried out on genomic DNA extracted from whole blood using a DNA extraction kit (Fermentas, Burlington Ontario, Canada) after obtaining informed consent. We amplified polymerase chain reaction (PCR) using an i-Cycler thermal cycler (Bio-Rad, Hercules, California) in all 26 exons of *SCN1A*. PCR fragments were heat denatured at 94ºC for 7 minutes and slowly cooled to room temperature to form heteroduplex products which were analysed by denaturing high performance liquid chromatography (DHPLC). To identify and confirm the mutation, direct sequencing analysis was performed with a BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), a genetic analyzer (ABI Prism 310; Applied Biosystems), and DNA Sequencing Analysis Software (Applied Biosystems). In case where a *SCN1A* mutation was detected, the appropriate amplication from parental DNA was tested by DNA sequencing to distinguish between de novo and familial variants.

The Cases

In family of patient 1 (**Figure 1a**), four members over four successive generations had febrile seizure (FS) and febrile seizure plus (FS+). Other family members had no history of febrile or afebrile seizures. However, there was no family history of autism. In family of patient 2 (**Figure 1b**), three members over three successive generations had FS in a pattern strongly suggestive autosomal dominant inheritance. There was no family history of autism either. In family of patient 3 (**Figure 1c**), nine members over four successive generations had FS, with absence of family history of autistic spectrum disorder. In family of patient 4 (Figure 1d), there were no family histories of FS, epilepsies, and autism.

In family pedigree of patient 1, mutational analysis of SCN1A identified in this family was a missense mutation, c.4834G>A (p.V1612I), which was only found in the patient. This mutation was not found in parents and his brother. We identified SNPs c. 383+66T>C, c.603-91G>A, c.603-106G>T and c.1028+21T>C in this patient as well. In family pedigree of patient 2, no mutation or single nucleotid polymorphism (SNP) was detected in this case. In family pedigree of patient 3, no mutation was identified. We only found SNPs c. 383+66T>C, c.603-91G>A, c.603-106G>T and c.1028+21T>C

(a) Family A
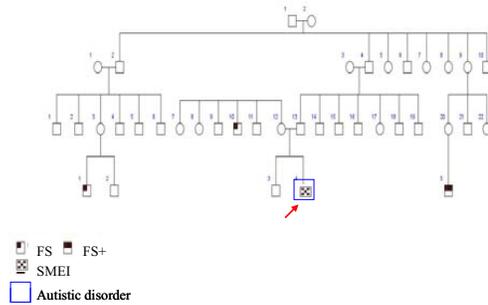
**(b) Family B**
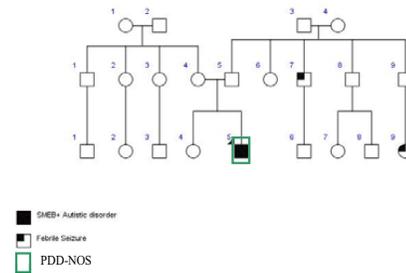
(c) Family C
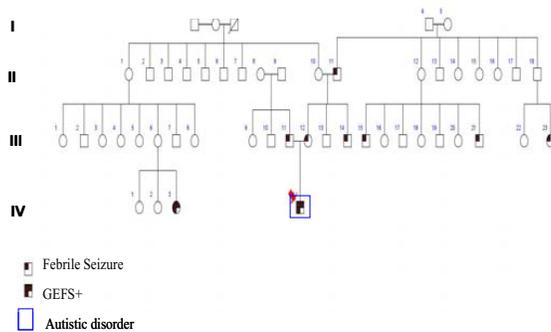
(d) Family D
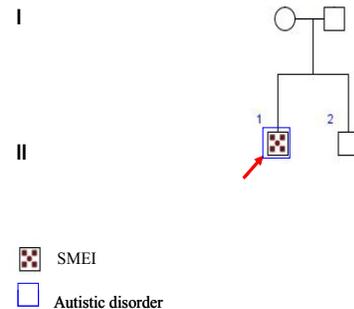
Figure 1. Pedigree of Indonesian families with autosomal dominant epilepsy with febrile seizures plus

in this patient. In family pedigree of patient 4, no mutation was observed. We only found SNPs c. 383+66 T>C, c.603-91G>A, c.603-106G>T and c.1028+21T>C in this case as well and c.2173G>A and c.2176+44C>T.

Patient 1 (RP)

A 5-year and 10 months old boy exhibited febrile generalized tonic-clonic convulsion at 7 months. After the first occurrence, such febrile seizures recurred several times until he reached three years old. Thereafter, he suffered various types of seizures including generalized tonic clonic seizures (GTCS), myoclonic seizures, left-sided hemiconvulsions, absences and focal convulsions without fever. His seizures were too intractable to be controlled with anticonvulsants such valproate acid (VPA),

oxcarbazepine, phenytoin or clonazepam. As for psychomotor development, he presented normal gross motor milestones, but delayed speech development. He had difficulty in social interaction, no eye contact, flapping, and was hyperactive. EEG showed slowing background activity (**Figure 2a**). On the basis of these clinical features, he was diagnosed as having SMEI and autistic spectrum disorder.

Patient 2 (RH)

A 1-year and 8 months old boy had his first fever-induced status epilepticus (SE) at age 8 months and valproic acid was initiated. Although clonazepam was added for his seizures, they persisted frequently with or without fever and repeated frequently. He had many types of seizures such as tonic-clonic, tonic and

EEG Patient 1
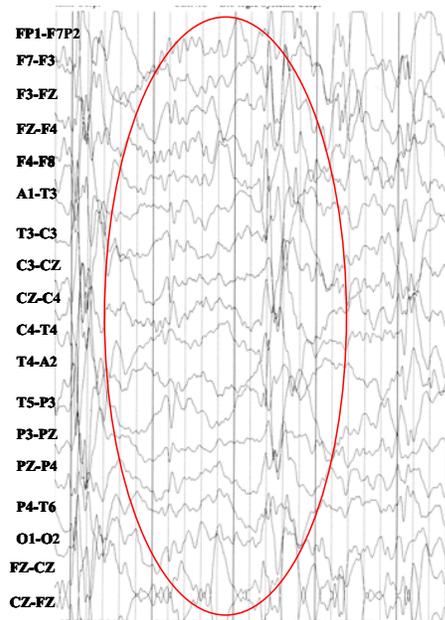
EEG Patient 2
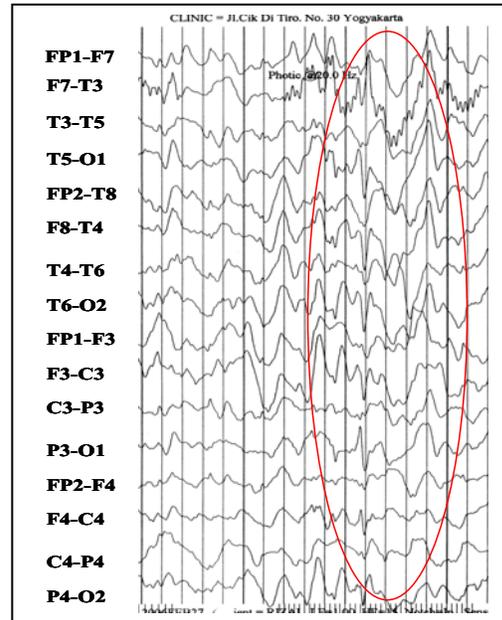
EEG Patient 3
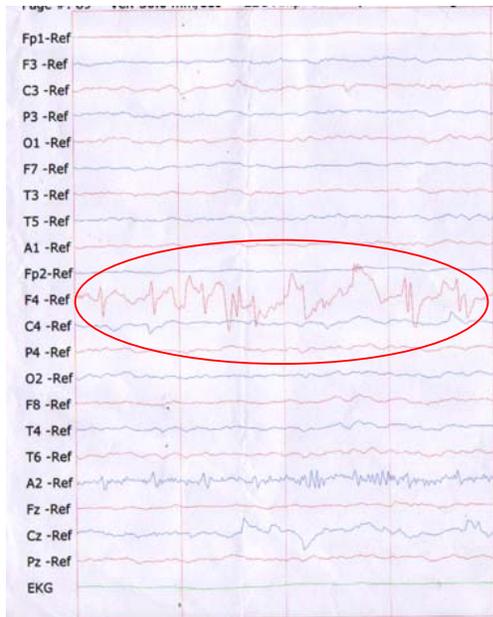
EEG Patient 4
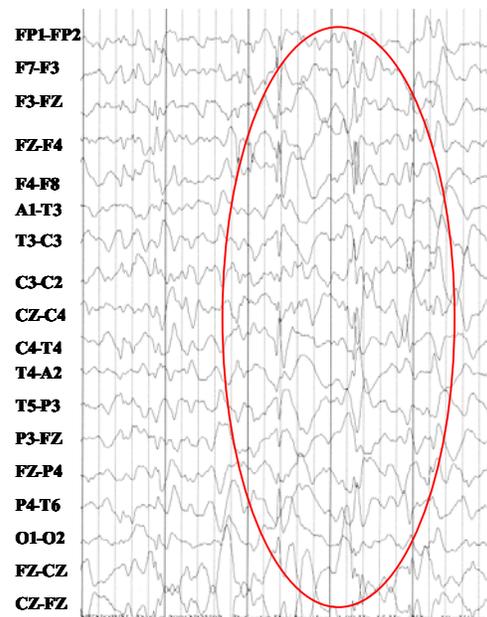
Figure 2. (a) Interictal EEG of the patient 1, revealed spike-and-wave complexes and polyspikes (b) Interictal EEG of the patient 2, revealed spike-and-wave complexes. (c) Interictal EEG of the patient 3, revealed spike-and-wave in the right frontal. (d). Interictal EEG of the patient 4, showed spike-and-wave complexes.

myoclonic. At that time, his parents also complained that their son could not used words to communicate, had stereotyped and repetitive motor mannerism such as hand flapping, no response to a call; however, an eye contact is good. EEG revealed within normal limit (**Figure 2b**). He was diagnosed with SMEB and pervasive developmental disorder- not otherwise specified (PDD-NOS).

Patient 3 (MNA)

A 2-year-old boy was born with normal delivery and his motor development was normal. At 7 months of age, he had general tonic clonic seizures with high fever for a few minutes. After the first seizure, he had seizures four times with or without fever up to 2-years old. EEG exhibited spike-and-wave in the right frontal (**Figure 2c**). He was treated with VPA and became seizure free. However, at 2 years old, he could not speak, had lack of social or emotional reciprocity, failed to develop peer relationships with his friends, had no eye contact, and showed hand flapping. He was diagnosed with GEFS+ and autistic disorder. The clinical manifestation of autism persist until this time.

Patient 4 (MBS)

A 7-year-old boy was born after a normal pregnancy and delivery. At 3 months of age, he had hemi-convulsion with high fever for few minutes. The second seizure appeared at 9 months old with myoclonic type and the myoclonic seizure and continued until 1 year of age. After 1 year old the seizure occured more frequently, 3-4 times per month

with or without fever and type of fever changed to GTCS. EEG showed spike-and-wave complexes (**Figure 2d**). He was treated with phenytoin by a pediatrician; however, the seizure did not improve. At 6 years and 4 months old he came to our hospital because the seizure persisted and his parents complained that their son could not speak, failed to develop peer relationships with his friends, had no eye contact, hand flapping, lack of social or emotional reciprocity and ataxia. He was diagnosed with SMEI and autistic disorder. At that time, VPA was added to treat the seizure. Thereafter, the patient and his family moved to other island. However, his grandfather still consulted us and told that the signs and symptom of autism and seizure persist until now. He studies in a special school.

Results

Four patients, all boys with the diagnosis of GEFS+ in one patient, SMEB in one patient and SMEI in two patients. Three patients suffered from autistic disorder and one patient developed PDD-NOS (**Table 1**).

The four pedigrees of the patients are presented in **Figure 1**. Three individuals in the family of patient

Table 1. Mutation and SNPs in the *SCN1A* gene

No	Diagnosis	Location in gene	DNA change	Hetero/ homozygote	Amino acid substitution	Inheritance
1.	SMEI & Autistic disorder	Intron 2	c.383+66 T>C	heterozygote	--	--
		Intron 4	c.603-91 G>A	heterozygote	--	--
		Intron 4	c.603-106 G>T	homozygote	--	--
		Intron 7	c.1028+21T>C	heterozygote	V1612I	--
		Exon 25	c.4834 G>A	heterozygote	--	De novo
2.	SMEB &	Intron 12	2177-38C>A(rs)	homozygote	--	--
		Intron 12	2177-12delT(rs)	heterozygote	--	
3.	PDD-NOS GEFS+ & Autistic disorder	Intron 2	c.383+66T>C	heterozygote	--	--
		Intron 4	c.603-9 G>A	heterozygote	--	--
		Intron 4	c.603-106 G>T	homozygote	--	--
		Intron 6	c.965-21C>T	homozygote	--	--
		Intron 7	c.1028+21T>C	heterozygote		
4.	SMEI & Autistic disorder	Intron 2	c.383+66 T>C	heterozygote	--	--
		Intron 4	c.603-91 G>A	heterozygote	--	--
		Intron 4	c.603-106 G>T	homozygote	E725K	--
		Intron 7	c.1028+21 T>C	heterozygote	--	--
		Exon 12	c.2173 G>A	heterozygote		--
		Intron 12	c.2176+44 C>T	heterozygote		--
		Intron 12	c.2177-12delT	heterozygote		

1 and patient 2 were classified as affected GEFS+ spectrum, whereas nine individuals in family of patient 3 and only one patient in family of patient 4 were affected. Electroencephalography of all patients showed abnormality (**Figure 2**).

Nineteen additional variants (four homozygote and 15 heterozygot) were detected, including 18 single-nucleotide polymorphisms (SNPs) and 1 missense mutation (Table 1). The same SNPs, c.383+66T>C, c.603-106G>T and c.603-91G>A and c.1028+21T>C were observed in three patients, whereas we detected c.2177-12delT (rs) in two patients, and c.965-21 C>T, c.2173G>A, c.2176 +44 C>T, and 2177-38C>A were found in one patient each. A base substitution of c.4834, G →A was detected in the DNA derived from patient 1 (Figure 3).

Nucleotide sequences of exon 25 in SCN1A are shown partially. There were G → A substitutions in the DNA derived from the proband; however, this mutation was not detected in the DNA derived from his mother, father and controls. As a result, the codon that involves the mutation was changed from GTC to ATC, which corresponded to a substitution of 1612th amino acid in Nav 1.1, valine to isoleucine (V1612I).
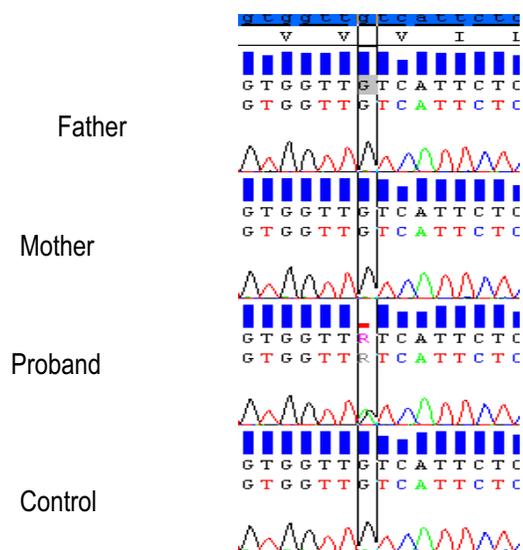

Figure 3. The mutation detected in the proband.

Discussion

The correlation among autism, neurologic dysfunction and epilepsy suggests an underlying encephalopathy presenting with a combination of neurologic abnormalities, including clinical epileptiform activity.[18] In our study, the onset of febrile seizure was earlier, in the first year of life in all patients and the clinical manifestation of autism appeared after the patients suffered from febrile seizure or epilepsy. This condition was consistent with the study by Levihson[18], in 2007, which reported that epilepsy itself is a risk factor for autism, independence of other central nervous system dysfunction. Gillberg and Steffenburg[19], in 1986, also mentioned that epilepsy most commonly occurs in autistic children before the age of 5 years and recurs after 10 years of age. All of the patients in this study were male, whereas some reports have indicated that epilepsy is more common in female autistic adolescents than in males,[20-22] and an other report wrote that the occurrence of epilepsy in autism was the same for both genders.[23]

Hara[23], in 2007, reported that the occurence of febrile seizures in the austistic individuals was 14% in total. The report appears to be slightly higher than in the general population. Unfortunately, such data are not available in Indonesia. In our study, we had four patients only, with history of febrile seizure. On the other hand, the prevalence rate of childhood epilepsy is 0.4-1%, compared with 42% in autism.[21,24]

Electroencephalography abnormalities and epilepsy in children with autism were the first recognized evidence of the neurobiologic etiology of autism. The definition of EEG abnormality in autism patient was broader than currently accepted and included not only epileptiform features, such as spike and spike-wave discharges but also less clearly abnormal features such as diffuse theta, low-voltage fast and amorphous background.[25] In our study, one patients exhibited spike-and-wave complexes, other patient revealed slowing background activity and one patient showed normal EEG. The EEG result indicated epilepsy was stronger than autistic disorder. On the other hand, Lehvison18 (2007) mentioned that the EEG result, in particular a sleep-deprived EEG with appropriate sampling of slow-wave sleep, is probably appropriate if there is a history of clinical seizures or suspicion of subclinical seizures, and a history of

regression at any age.

Considering the essential role of sodium channels in the central nervous system and the sensitivity of neuronal firing patterns to subtle mutations in these channels, sodium channel mutations may affect cognitive and emotional functions.[26] Osaka et al[8], 2007, reported that no autistic spectrum and other psychiatric illnesses had not been reported in families with FS plus, intractable childhood epilepsy with generalized tonic clonic seizures (ICEGTC) or SMEI. Weiss et al[7] (2003) screened *SCN1A* autistic patients from Autism Genetic Research Exchange Families. They found mutations in six patients. These were missense mutations, R542Q, I1034T, F1038L in *SCN1A* gene, and R1902C in *SCN2A* among 117 families. They were not found in control tested. R542Q and F1038L mutations were shared by siblings. They hypothesized that, eventhough *SCN1A* mutations may not be major determinants of genetic abnormalities in autism, the combined effect of these mutations may predispose to these psychiatric diseases.[7,27]

In our previous study[28], we reported two novel missense mutations of SCN1A gene in Indonesian patients with SMEB and SMEI , including one patient in the present study (patient 1). We found missense mutation V1612I in patient 1 with clinical manifestation of SMEI and autism. The mutation was not observed in his parents and brother. However, the genetic analysis to identify the mutation had not been performed yet in his other family members who had history of febrile seizure. None of his family members showed clinical manifestation of autism. We did not observe the same mutation in 100 people Indonesian and Malaysian controls. In this case, we wonder the mutation may result in abnormalities in both diseases. We also observed four same SNPs in three other patients c. 383+66 T>C , 603-91 G>A, c.603-106 G>T, c.1028+21T>C and SNP c.965-21 C>T, c. 2173 G>A and 2176+44C>T in one patient. These polymorphic sodium channel variants may contribute to psychiatric disorders such as autism or many types of epilepsy.

In conclusion, we reported in this study the first autistic patient who also suffered from SMEI with a missense mutation in the *SCN1A* gene in the Indonesian population. To ensure that *SCN1A* gene has a role of mutation in autistic patients, we recommend performing gene analysis in children who suffer from autism only in future studies.

Acknowledgments

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