Polish Annals of Medicine

journal homepage: https://www.paom.pl

Original article

SCN1A gene variations in epilepsy and migraine patients in Aceh, Indonesia

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ARTICLE INFO

Article history
Received 2 January 2017
Accepted 30 October 2017
Available online 24 January 2018

Keywords
Epilepsy
Gene variation
Migraine
Mutation
SCN1A

Doi
10.29089/2017.17.00001

ABSTRACT

Introduction: Mutation of the sodium voltage-gated channel alpha subunit 1 (*SCN1A*) gene is an important cause of genetic epilepsy and familial hemiplegic migraine. However, data related to genetic variations of *SCN1A* in Indonesia are limited.

Aim: To identify *SCN1A* gene variation in idiopathic epilepsy and common migraine patients in Aceh province, Indonesia.

Material and methods: A case-control study was conducted at Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia from 1 March to 30 August 2015. Gene variation analysis of exon 26 of the *SCN1A* gene was conducted in 33 patients with idiopathic epilepsy, 33 patients with common migraine and 30 controls using polymerase chain reaction and direct sequencing.

Results and discussion: *SCN1A* gene variations were identified in two partial secondary generalized epilepsy patients. In 1 patient, four silent mutations at nucleotide positions A4440T (Leu1480Leu), T4443C (Leu1481Leu), A5046G (Leu1682Leu) and C5121T (Asn1707Asn) were identified. One silent mutation at position G5505A (Glu1835Glu) was found in another patient. No gene variation was identified among controls and common migraine patients.

Discussion: This study is the first report on genetic variations of the *SCN1A* gene in adult patients with idiopathic epilepsy and common migraine in Indonesia. However, the association between these genetic variants and epilepsy needs to be clarified.

Conclusions: Five genetic variations in exon 26 of *SCN1A* were identified in 2 patients with partial secondary generalized epilepsy in Aceh, Indonesia.

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1. INTRODUCTION

Voltage gated sodium channels (NaV) are essential for the generation of neuron excitability. These channels are the target of antiepileptic drugs, and mutations of their genes are responsible for genetic epilepsy.[1] Sodium voltage-gated channel alpha subunit 1 gene (*SCN1A*), which encodes the alpha subunit of the sodium channel termed NaV1.1, is one of the most clinically relevant epilepsy genes with hundreds of identified mutations. Genetic variations in *SCN1A* have been reported to be associated with epilepsy syndromes characterized by various phenotypes including generalized epilepsy with febrile seizure plus (GEFS+),[2] Dravet syndrome,[3–5] severe myoclonic epilepsy in infancy (SMEI),[6,7] intractable childhood epilepsy with generalized tonic-clonic seizures (a variant of Dravet syndrome without myoclonus)[8] and cryptogenic epilepsy.[9]

Mutations of the *SCN1A* gene are also associated with familial hemiplegic migraine (FHM).[10–12] FHM is a rare severe autosomal dominant inherited subtype of migraine with aura characterized by hemiparesis during the attacks. Several missense substitution mutations have been identified to be associated with FHM subtypes, these include pure FHM, migraine with or without aura, and mixed phenotypes with seizures and migraine.[13] At least five mutations in the *SCN1A* gene have been identified in individuals with FHM-type III.[13] Each of these mutations changes a single protein building block (amino acid) in the NaV1.1 channel, which alters the structure of the channel.[13] This increases the flow of sodium ions into neurons and triggers the cell to release more neurotransmitters.[13]

Although several studies have been conducted to identify variations of the *SCN1A* gene among epilepsy and migraine patients from different countries, no research has been conducted in Aceh province, Indonesia. Therefore, this study sought to provide data from Indonesia regarding *SCN1A* gene variations in epilepsy and migraine patients.

2. AIM

This study was conducted to identify variations of the *SCN1A* gene among patients with idiopathic epilepsy and common migraine in Aceh, Indonesia.

3. MATERIALS AND METHODS

3.1. Ethical approval

The protocol used in this study was approved by the Ethics Committee of the Faculty of Medicine, Sumatera Utara University, Medan, Indonesia in compliance with the national legislation and the code of ethical principles for medical research involving human subjects of the World Medical Association (Declaration of Helsinki, No. 131/KOMET/FK USU/2015). Written informed consent was obtained from each subject prior to enrolment. Participation in this study was voluntary, and no incentives were provided.

3.2. Study setting and subjects

A case-control study was conducted at the Neurology Polyclinic of the Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia from 1 March to 30 August 2015. The cases were adult patients with confirmed idiopathic epilepsy (generalized or partial) and common migraine patients, while controls were healthy individuals or patients with mild traffic accident injuries. Diagnosis and classification of epilepsy and migraine were conducted based on criteria of the International League Against Epilepsy (ILAE)[14,15] and International Classification of Headache Disorders (ICHD),[16] respectively. Epilepsy patients who were unable to communicate appropriately due to other underlying clinical problems, such as mental retardation, aphasia or dementia, were excluded. Upon admission, demographic data, clinical signs and symptoms and neurologic status were assessed and venous blood samples were collected under hospital Standard Operation Procedures. The blood samples were kept at –20°C until analyzed.

3.3. DNA extraction and SCN1A genotyping

DNA extraction was carried out at the School of Medicine, Syiah Kuala University, Banda Aceh, Indonesia as described previously.[17] Amplification of exon 26 of the *SCN1A* gene was conducted at the Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia. The sequencing of amplified gene was conducted in 1st BASE Pte. Ltd. Laboratory, Singapore, with the same platform as described previously.[18] Briefly, DNA from peripheral blood leukocytes was extracted using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Exon 26 of *SCN1A* was amplified by polymerase chain reaction (PCR) using three pairs of primer (Table 1). The length of amplicon of fragments A, B and C were 587, 470 and 593 base pairs, respectively. Amplification was performed for 35 cycles (denaturation at 95°C for 1 minute, annealing at 60°C for 2 minutes and extension at 72°C for 1 minute) in a total 30 μL PCR mixture containing 2 μL DNA template, 15 μL PCR Master MixGo Taq Green Promega (1X buffer PCR, 150 nM dNTP, and 0.5 U Taq DNA polymerase) (Promega, Madison, WI, USA), 11 μL nuclease free water, and 2 μL primer mix (1 μL forward primer and 1 μL reverse primer). PCR products

Table 1. The primers used to amplify exon 26 of the *SCN1A* gene.

Fragment	Forward (5' to 3')	Reverse (5' to 3')
A	AGGACTCTGAACCTTACCTTG	TACATGTTCACCACAACCAGG
B	TAACCCTGGAAGCTCAGTTAA G	TGATTGGCTGATAGGAGACCTT
C	TTGCTTTTACAAAGCGGGTTCT	GTTTGCTGACAAGGGGTCAC

Table 2. Subject characteristics of patient and control groups.

Characteristics		Epilepsy $n = 33$		Migraine $n = 33$		Control $n = 30$		P value*
		n	%	n	%	n	(%)	
Age group	< 20 years	7	21.21	4	12.12	2	6.67	0.001*
	21–30 years	13	39.39	3	9.09	4	13.33	
	31–40 years	8	24.24	12	36.36	11	36.67	
	41–50 years	5	15.15	10	30.30	9	30.00	
	> 50 years	0	0.00	4	12.12	4	13.33	
Gender	Male	17	51.52	6	18.18	18	60.00	0.002**
	Female	16	48.48	27	81.82	12	40.00	
Education	Primary or junior high school	5	15.15	7	21.21	4	13.13	0.292**
	Senior high school	21	63.64	10	30.30	14	46.67	
	University diploma degree	2	6.06	6	18.18	3	10.00	
	University graduate or higher	5	15.15	10	30.30	9	30.00	
Occupation	Civil servant	3	9.09	9	27.27	13	43.33	0.001***
	Entrepreneur	7	21.21	8	24.24	10	33.33	
	University student	12	36.36	5	15.15	3	10.00	
	Housewife	5	15.15	11	33.33	4	13.33	
	Unemployment	6	18.18	0	0.00	0	0.00	
Ethnic	Acehnese	29	87.88	29	87.88	30	100.00	0.294***
	Non-Acehnese	4	12.12	4	12.12	0	0.00	

Comments: * Calculated using Mann-Whitney test, * Calculated using χ^2 test, * Calculated using Likelihood ratio.

were electrophoresed through ethidium bromide-stained 2% agarose gels. The PCR products were then sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

3.4. Statistical analysis

Differences in distributions of the data between case and control groups were analyzed using the Mann-Whitney test, χ^2 test, or likelihood ratio as appropriate for the type of data. Two-sided testing was employed and $P \leq 0.05$ was designated statistically significant. The data were analyzed using the Statistical Package for the Social Sciences (SPSS for Windows v. 15, Chicago, IL).

4. RESULTS

In this study, the exon 26 of the *SCN1A* gene of 33 idiopathic epilepsy patients (4 generalized epilepsy and 29 partial secondary generalized epilepsy), 33 common migraine patients (16 migraine without aura and 17 migraine with aura) and 30 control individuals was analyzed. The characteristics of each of the patient groups and the control group are presented in Table 2. Clinical characteristics of the epilepsy and migraine groups are presented in Table 3.

Five gene variations in exon 26 of the *SCN1A* gene were identified in 2 epilepsy patients. Four gene variations were identified in a 32-year-old male with partial secondary gener-

Table 3. Characteristics of migraine and epilepsy groups.

Case	Characteristics	n	%
Migraine			
	Aura		
	With aura	17	51.52
	Without aura	16	48.48
	Headache attacks		
	Less than 12 days per year	3	9.09
	12-180 days per year	30	90.91
Epilepsy			
	Type of seizure		
	Partial seizures evolving to secondarily generalized seizure	29	87.88
	Generalized seizure	4	12.12
	Frequency of seizure		
	1-11 seizures per year	23	69.70
	Frequent seizure	10	30.30
	Aura		
	With aura	20	60.61
	Without aura	13	39.39
	Interictal EEG		
	Abnormal	21	63.64
	Normal	10	30.30
	Not available	2	6.06
	Level of stigma		
	Mild	4	12.12
	Moderate	29	87.88

alized epilepsy, and a single gene variation was identified in a 19-year-old female with partial secondary generalized epilepsy. The 4 variations in the first patient were mutations at nucleotide positions 4440, 4443, 5046 and 5121 causing silent mutations at codons 1480 CTA→CTT (Leu1480Leu), 1481 CTT→CTC (Leu1481Leu), 1682 CTA→CTG (Leu1682Leu) and 1707 AAC→AAT (Asn1707Asn), respectively (Figure 1A–C). In the second patient, a silent mutation at position 5505 causing a silent mutation at codon 1835 GAG→GAA (Glu1835Glu) was identified (Figure 1D). No gene variations were identified among the common migraine patients or controls.

5. DISCUSSION

This study is the first report on genetic variations of the *SCN1A* gene in adult patients with idiopathic epilepsy and common migraine in Indonesia. We found 5 silent mutations that were spread throughout exon 26 of the *SCN1A* gene in 2 epilepsy patients. Several studies have been conducted to show that *SCN1A* mutations are associated with epilepsy[3–9] and migraine.[10–12] More than 100 epilepsy-associated mutations spread throughout the *SCN1A* gene have been reported.[19] A study found that 14.66% of 150 Italian pediatric probands with epilepsy carried *SCN1A* gene mutations.[2] Another study identified *SCN1A* mutations in 24 out of 29 patients with SMEI.[20] In this study, we found that 6.60% (2/33) of idiopathic epilepsy patients carried *SCN1A* gene variations, and none of the common migraine patients had gene variations.

Previously, a study in Indonesia identified 2 novel *SCN1A* mutations in Indonesian children with SMEI and borderline SMEI (SMEB).[7] These mutations were located at nucleotide 4834 (c.4834G>A) in exon 25 leading to substitution of valine with isoleucine at amino acid position 1612 (p.V1612I), and at nucleotide 5266 (c.5266T>G) in exon 26 leading to substitution of cysteine with glycine at amino acid 1756 (p.C1756G).[7] In our study, *SCN1A* gene variations were identified at exon 26 in 2 patients with partial secondary generalized epilepsy. The variations were 4 silent mutations at nucleotide positions A4440T (Leu1480Leu),

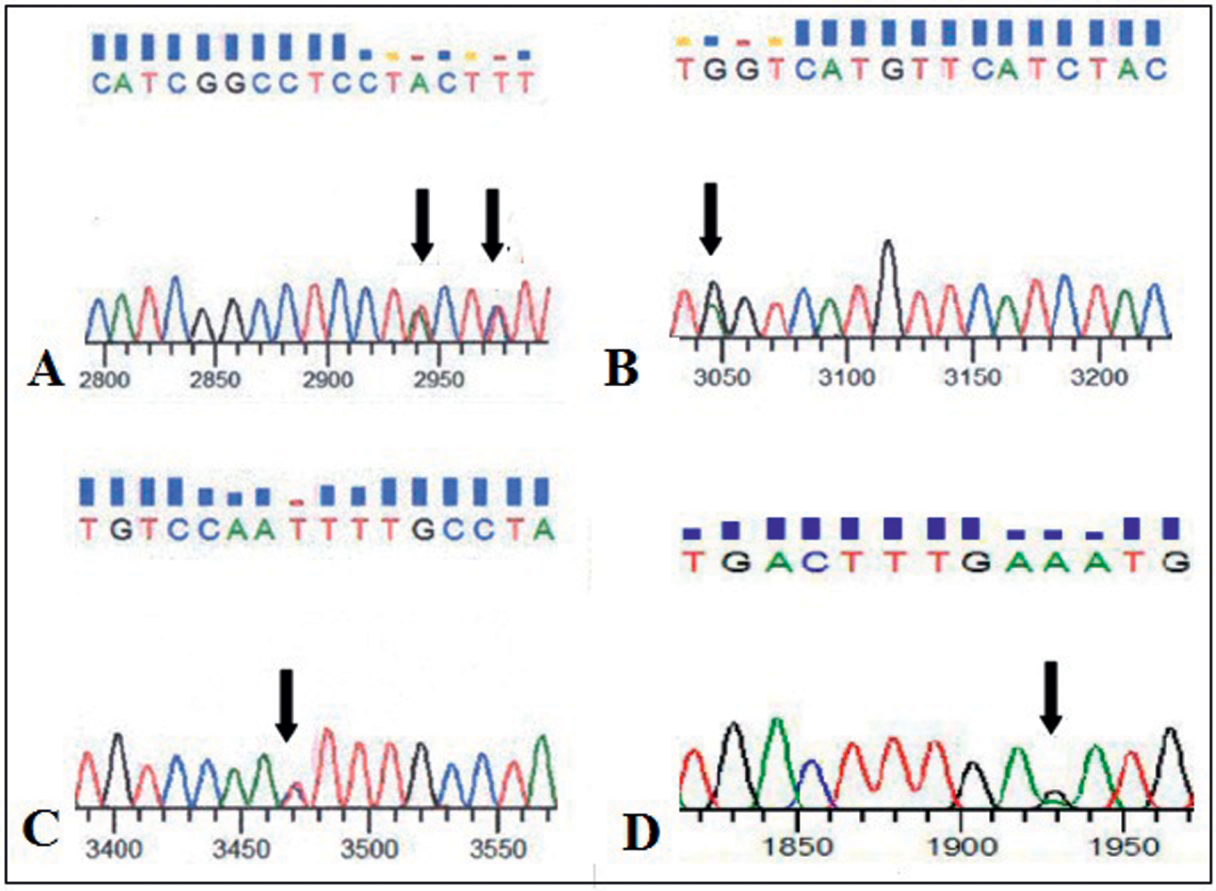

Figure 1. Sequencing electropherogram of *SCN1A* genetic variations in two patients: (A) The arrows show the genetic variations in the first patient at nucleotide positions 4440 and 4443 causing silent mutations at codons 1480 CTA→CTT (Leu1480Leu) and 1481 CTT→CTC (Leu1481Leu), respectively. (B–C) The arrows show the genetic variations in the first patient at nucleotide positions 5046 and 5121 causing silent mutations at codons 1682 CTA→CTG (Leu1682Leu) and 1707 AAC→AAT (Asn1707Asn), respectively. (D) The arrow shows genetic variations at nucleotide position 5505 causing a silent mutation at codon 1835 GAG→GAA (Glu1835Glu) in the second patient.

T4443C (Leu1481Leu), A5046G (Leu1682Leu) and C5121T (Asn1707Asn) in patient 1 and a silent mutation at position G5505A (Glu1835Glu) in patient 2. To the best of our knowledge, these variations have never been reported in Indonesia.

Interpretation of genetic results is challenging, especially in multifactorial diseases, such as epilepsies and the common migraine. Our study was unable to determine the association between these genetic variations and epilepsy for several reasons. First, the sample size in this study was relatively small. Second, the identified genetic variations were silent mutations that produced the same amino acid and therefore might not effect NaV1.1 channel configuration and function. Lastly, unlike some disorders where mutations are largely concentrated in 'hot spots' within the gene, mutations within *SCN1A* are widely distributed throughout the gene.[19,21] In this study, we did not investigate the genetic variations that might have existed within other exons. Therefore, the association of genetic variations in other exons with epilepsy needs to be further clarified.

6. CONCLUSIONS

For the first time, this study has identified five genetic variations of SCN1A in 2 patients with partial secondary generalized epilepsy in Aceh, Indonesia. No genetic variation of the SCN1A gene was identified in common migraine patients.

Conflict of interest

None declared.

Acknowledgements

We would like to express our sincere appreciation to all subjects for their participation. This study was supported by the Indonesian Directorate of Research and Community Service, grant number 025/SP2H/LT/DRPM/II/2016. We would like to thank to all participants in this study.

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