

Sodium Voltage-gated Channel Alpha Subunit 1 gene (SCN1A) among Epileptic Sudanese Patients

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Abstract

Background: Epilepsy is a pathological condition characterized by recurrent, unprovoked, epileptic seizures the SCN1A gene represents one of the most commonly mutated human epilepsy genes.

Aim: The study aimed to investigate mutated SCN1A gene in patients with idiopathic epilepsy. It also determined the serum level of sodium, potassium.

Methods: the current study is a cross-sectional study that had been performed at Sheikh Mohamed Khair Centre, Banat, Omdurman, and National Centre for Neurological Sciences (NCNS) Khartoum state, during the period from November 2016 to February 2019. A total of 100 patients were enrolled in this study. Sodium and Potassium concentrations were obtained by the full automated Easylyte instrument (Na/K analyzer) and the SCN1A gene which located on chromosome 2 of humans, using conventional PCR. The data were analyzed using SPSS version (25) and bioinformatics tools were used to analyze sequencing results.

Results: One hundred subjects were recruited in this study, (47% males and 53% females) diagnosed with epilepsy. Patients aged between 18-40 years old were the highest category with a 55%. Onset of seizure was in age group of less than 5 yrs were 50% of the patients, in age group 5-10 yrs 28% and more than 10 yrs were 22%. Ninety percent of the patients had a Family history with epilepsy while the rest of them (10%) had not. Generalize epilepsy presented in 68% focal to bilateral in 26% and focal in 6 patients two of them (2%) developed impairment. Serum sodium and potassium analysis showed normal results, mean 134.82 mg/dl, 3.65 mmol/l, respectively. All patients were tested positive for SCN1A gene.

Conclusion: Genetic mutations have an effective role in developing epilepsy. AT deletion in SCN1A gene, indirectly affects gamma amino butyric acid function.

Key words: SCN1A gene, epilepsy, neurological disorders, Sudanese, serum electrolytes

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Introduction

Epilepsy is a pathological condition characterized by recurrent, unprovoked, and epileptic seizures (1). It affects approximately 42 million people worldwide and it is the most common heterogeneous neurological disorder with distinct symptoms, etiology, prognosis and

treatments (2). Nearly 70% of patients with epilepsy lack an obvious pathogenetic cause. Genetic is believed to play an important role in the cause of epilepsy (3). 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 (4). The SCN1A gene codes for the alpha subunit of the neuronal voltage-gated sodium ion channel, type 1 (NaV 1.1) (5). It is located on chromosome 2 of humans, and is made up of 26 exons spanning a total length of 6030 nucleotide base pairs (bp) (6). It is expressed in the central and peripheral nervous systems and in cardiomyocytes (6). Voltage-gated sodium channels are an important group of ion channels, which play an important role in generating action potential and depolarization of the neurons (7). Action potential is the electric signal that travels along the axon and sends information to other neurons (8). It seems logical that mutations in voltage-gated sodium channels would cause epilepsy, because these channels are in part responsible for controlling electrical excitability (9). This study investigated SCN1A mutation in patients with idiopathic epilepsy in addition to sodium and potassium level measurement.

Materials and Methods

Study type and population: The current study is a cross-sectional study that had been performed at Sheikh Mohamed Khair Centre, Banat, Omdurman, and National Centre for Neurological Sciences (NCNS) Khartoum state, during the period from November 2016 to February 2019. Ninety nine patients were enrolled in this study. Only the patients who were diagnosed having idiopathic epilepsy were included in this study, the rest of them were excluded.

Sample collection and preparation: Blood samples were drained from each patient in two containers, (EDTA) and LI-heparin, the EDTA samples were processed for DNA extraction, and the heparinized samples were used for Sodium and potassium estimations.

Data collection: Clinical and demographic data were collected in a predesigned questionnaire. The demographic and clinical data relating to each patient were registered (age, gender, family history, class of seizure, onset of seizure).

Biochemical analysis

Serum was separated by centrifugation (Hettich Zentrifuge EBA200, Kirchleingern, Germany) at 3,000 RPM for 5 minutes and then stored at -20°C. Sodium and Potassium concentrations were obtained by the full automated Easylyte instrument (Na/K analyzer).

Molecular Genetic Analysis

Deoxyribonucleic acid (DNA) was extracted from whole blood using QIAGEN® DNA extraction kits (vacuum protocol). For polymerase chain reaction, 20 µl of ready-made MM (4 µL of 5× Firepol® Master Mix (Solis Bio-Dyne, Tartu, Estonia), 1 µL forward primer, 1 µL reverse primer, and 14 µL distilled water), and 2 µl of DNA were added into each PCR tube. Primers for SCN1A gene were 5'-TACCCTGTTCCGAGTGATCC-3' forward primer and 5'-GCTGTTGCCAAAGGTCTCAA-3' reverse primer were used for amplification, then the amplified PCR products (22 µL) were separated using 2% gel electrophoresis and then

the separated DNA was visualized using UV light.

DNA sequencing

Polymerase chain reaction products were transferred to china for sequencing at BGI solutions co.LTD.

Statistical Analysis

Analyses was done using Microsoft Office Excel and Statistical Package for Social Science Program 2010 SPSS version 25) for sequencing analysis, Bioinformatics tools were used to analyze sequencing results.

Results:

100 subjects were recruited in this study, (47% males and 53% females) diagnosed with epilepsy. Patients aged between 18-40 years old were the highest group with a 55%, followed by less than 18 years (32%), 41-65 years (11%) and more than 65 years were only 2%. Onset of

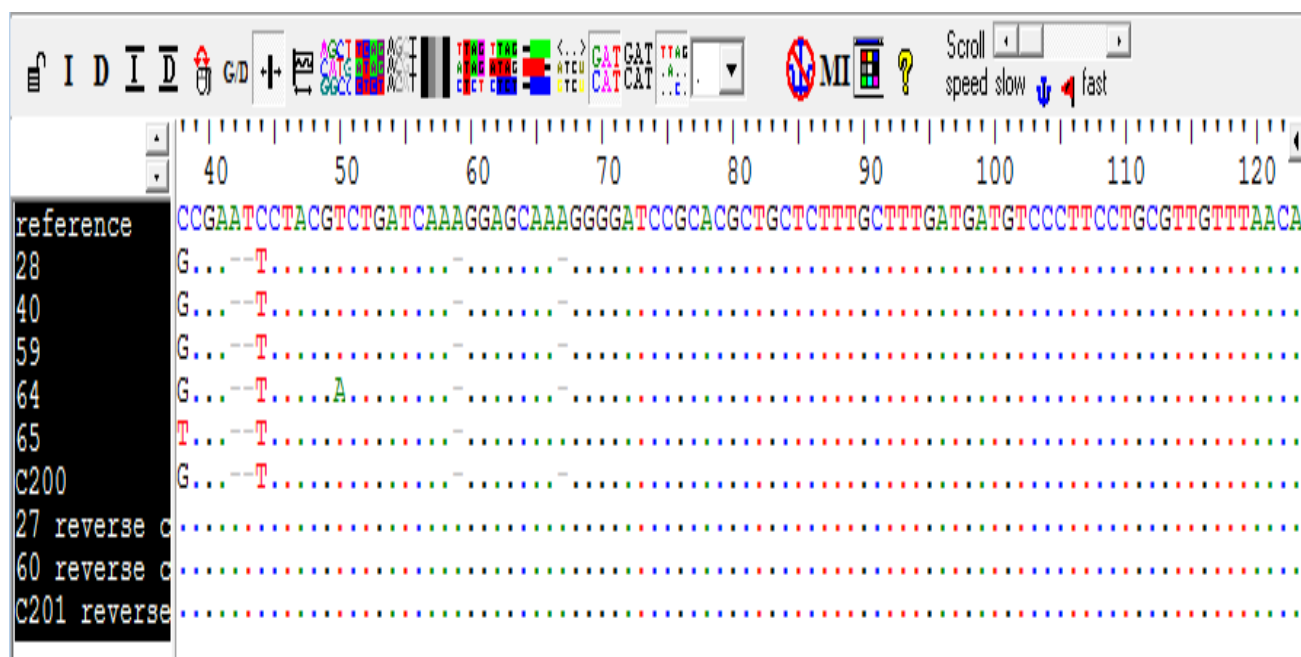
seizure was in age group less than 5 years were 50% of the patients. Ages between 5-10 years old was 28% and more than 10 years were 22%. Ninety percent of the patients had a Family history with epilepsy while the rest of them (10%) had not. Generalize epilepsy presented in 68%, focal to bilateral in 26% and focal in 6 % of the patients two of them (2%) developed impairment. Serum sodium and potassium analysis showed normal results, mean 134.82 mg/dl, and 3.65 mmol/l, respectively. The sequencing findings of SCN1A gene showed that, C > G (chr2:166848853) was detected in 57% of the samples and C > T in 14%, with splice site effect. Also A > T deletion with frame shift mutation (chr2:166848848) was appeared in 71% of the patients with splice site effect.

Table (1): The demographic data

		Count	Column N %
Age	More than 65	2	2.0%
	Less than 18	32	32.0%
	18-40	55	55.0%
	41-65	11	11.0%
Gender	Female	53	53.0%
	Male	47	47.0%
Onset of seizure	Less than 5 yr	50	50.0%
	More than 10 yr	22	22.0%
	5-10 yr	28	28.0%
Class of seizure	Focal	4	4.0%
	Focal to bilateral	26	26.0%
	Focal with impairment	2	2.0%
	Generalize	68	68.0%
Family history	No	90	90.0%
	Yes	10	10.0%

Table (2): Descriptive statistics (Mean±S) of chemicals

	Mean	Standard Deviation	Reference values
Na	134.82	7.95	135 - 145
K	3.65	.40	3.5 - 5

**Figure (1):** Sequence alignment of SCN1A gene of sample against reference sequence

Discussion

Epilepsy is a most common serious neurological disorder and is one of the world's most prevalent non-communicable diseases. Idiopathic (genetic) generalized epilepsies (IGEs) are common epilepsy syndromes. Regarding onset of epilepsy, this study showed that 50% of the patients had their onset of the disease at an age less than 5 years old. This was in favor to Ali A. Asadi's study only 15.2% were four years and under at the time of disease onset (10) though there is increasing evidence for the existence of IGE beginning beyond the third decade (11). In this study, females outnumbered males (53% females, 47% males). Similar findings were in Ali A.

Asadi study (females (57%), (43%) were males. Brain, among many other human tissues and organs, may be influenced by electrolyte disturbances. Seizures are often seen in patients with sodium disorders, especially, hypocalcemia, hyponatremia, and hypomagnesemia (12). numerous reports suggested that the body electrolytes (sodium (Na⁺), potassium (K⁺) and calcium (Ca²⁺) play a vital role in seizure condition to develop (13, 14). patient in the present study had sodium and potassium levels in the normal ranges .this might be because samples were collected when patients were in stable status as most of them were already under treatment. SCN1A is the most clinically relevant

gene for a large spectrum of epilepsy phenotypes and the search for a mutation in the SCN1A gene is the first widely accepted step in DNA diagnosis of patients assumed to have idiopathic epilepsy. Sequencing results showed that 71% of the sequenced samples had AT deletion chr2:166848848_166848849 resulting in amino acid substitution Isoleucine 1646 Proline. At the same exon (exon 26) analysis in patients with GEFS+2 identified a nucleotide substitution, G4943A, that results in the amino acid substitution Arg1648His (15). A substantial body of clinical data supports that high CNS L-proline may cause neuronal dysfunction by interfering with native neurotransmitter systems (16). In this regard, it was noted that the chemical structure of L-proline closely resembles that of GABA, suggesting pathological levels of L-proline might specifically disrupt normal GABA-ergic function(17)Lproline is a GABA-analogue, it's accumulation in the cytosol of GABA-ergic neurons causes competitive inhibition of glutamic acid decarboxylase (GAD) leading to deficient γ -Aminobutyric acid (GABA) production (17). GABA is recognized as the main inhibitory neurotransmitter in the cerebral cortex (18).It maintains the inhibitory tone that counterbalances neuronal excitation. When this balance is perturbed, seizures may ensue.

Conclusion:

Genetic mutations have an effective role in developing epilepsy, AT deletion in SCN1A gene, indirectly, affects Gamma aminobutyric

acid function which is inhibition of neuronal activity.

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