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# Association of SCN1A Mutations with Epilepsy among Sudanese Patients

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## Abstract

**Background:** Genetics research of humans has established that a genetic basis contributes to the susceptibility to epilepsy for a majority of the cases. Although many epilepsies are secondary to injury or another illness, approximately 40% are idiopathic, meaning that the original cause is unknown. It is presumed that most idiopathic epilepsies result from genetic abnormalities, with the majority likely caused by mutations in multiple currently unidentified genes. However, research has revealed a growing number of single-gene mutations that cause epilepsy. **Objective:** To detect some of the genetic mutations which may cause idiopathic epilepsy. **Methods:** The current study is a cross-sectional study that had been performed at Sheikh Mohamed Khair center, Banat, Omdurman, and National Centre for Neurological Sciences (NCNS) Khartoum state, during the period 2016 to 2019. Ninety-nine participants were enrolled in this study. Demographic data were collected in a predesigned questionnaire blood samples were analyzed for biochemical and molecular tests. **Results:** Ninety-nine patients diagnosed with idiopathic epilepsy were recruited in this study. The most affected age group was 18 - 40 years accounted for 55% of patients. Females were the majority with 53%. Fifty percent of the patients had the first seizure at age less than 5 years. Ninety percent of the patients have no Family history with epilepsy. All sequenced samples showed genetic mutations, deletion mutation was detected in 71% of the samples. Bioinformatics tools detected a frameshift mutation in the chain of the amino acids. **Conclusion:** The current study detected deletion mutations in SCN1A gene (frameshift) can cause epilepsy by changing some amino acids with residues that can affect neuronal stability indirectly.

**Keywords**

Epilepsy, National Centre for Neurological Science, Sudan, SCN1A

## 1. Introduction

Epilepsy is one of the most frequent neurological disorders; the prevalence estimation for active epilepsy ranges from 0.2% to 4.1% [1]. It is a pathological condition characterized by repeated, unprovoked, epileptic seizures [2]. Diverse causes of epilepsy are commonly being recognized, such as structural, metabolic, or genetic causes; yet, the majority of epilepsies have unknown etiology [3]. It is not surprising that alterations in the ion channels may be a pathogenetic cause, and indeed, at least for some syndromes, epilepsies are increasing thought to be "channelopathies" either due to genetic mutations or as the endpoint of hitherto unknown pathological processes [4]. The voltage-gated $Na^+$ channels' primary role is the initiation of action potentials, making them critical determinants of neuronal excitability [5]; it consists of large α subunits which connect with further polypeptides, for instance, $\beta$ subunits to outline efficient voltage-gated ion channels [6]. Defects in sodium channels subunits make them susceptible to slow inactivation, *i.e.*, the membrane stays depolarized for a longer time, as a result, it can cause epileptogenesis and increase of seizures [7] [8]. It should be mentioned that changes in sodium channels had been detected in brain tissues of epileptic patients, suggestive of a potential role for sodium channels in the pathophysiology of epilepsy [9].

SCN1A encodes the NaV1.1 subunit expressed mostly in inhibitory GABAergic neurons and is enriched at the axon initial segment, implicating a role in the beginning and the spread of action potentials in these cells [10] [11]. It was first implicated in epilepsy in 2000 [12]. Several hundred mutations of SCN1A have been reported in epilepsy since first discovered, making it the most frequently known epilepsy gene [13].

This study aims to detect some of the possible causes of idiopathic epilepsy by analyzing a sequence of SCN1A gene in addition to calcium levels.

## 2. Materials and Methods

This study is a cross-sectional study that was performed at Sheikh Mohamed Khair centre, Khartoum state. This center is a primary health care center however idiopathic epilepsy diagnosis was provided by neurology physician depending essentially on the description of eyewitness to the seizure attack. Ninety-nine participants were enrolled in this study starting November 2016 to February 2019. Only Patients who were not diagnosed with idiopathic epilepsy and those who refused to patients were excluded from this study. Patients' demographic, and clinical data including age, gender, ethnicity, class of seizure, the onset of

seizure, frequency of seizure, seizure classification, precipitating factor, modality of treatment psychosocial aspects, concomitant illness, and family history were recorded in a predesigned questionnaire. Analyses were performed using Microsoft Office Excel 2010, and Statistical Package for Social Science Program (SPSS version 25). Blood samples were collected from each patient in two containers, EDTA container and LI-heparin, the EDTA samples were processed for DNA extraction, and the heparinized samples were used for calcium estimation.

### Biochemical Analysis

Plasma was separated by centrifuge (Hettich Zenterfuge EBA200, Kirchlengern, Germany) at 3000 RPM for 5 minutes and then stored at −20˚C. Calcium concentrations were obtained by semi-automated chemistry Analyzer (Mindary BA-88A).

## 3. Molecular Genetic Analysis

Extraction of deoxyribonucleic acid (DNA) was performed from whole blood samples using QIAGEN® DNA extraction kits (vacuum protocol).

SCN1A gene was amplified using polymerase chain reaction (PCR), in PCR tube 20 µL of readymade Master Mix (4 µL of 5× Firepol® Master Mix (Solis Bio-Dyne, Tartu, Estonia) was added to 1 µL forward primer, 1 µL reverse primer, and 14 µL distilled water), followed by 2 µL of DNA.

Primers used for amplification were 5-TACCCTGTTCCGAGTGATCC3 forward primer and 5'-GCTGTTGCCAAAGGTCTCAA3' reverse primer, then the amplified PCR products were separated using 2% gel electrophoresis. Then, separated DNA was visualized using UV light.

#### DNA Sequencing

Fifteen PCR products were sent to China for sequencing (BGI solutions co. LTD).

### Data Analysis

#### Sequencing Analysis

The sequencing results were analyzed using multiple bioinformatics software and tools. The nucleotides sequences of SCN1A gene were searched for sequences similarity using nucleotide BLAST NCBI (https://www.ncbi.nlm.nih.gov/) then subjected to multiple sequence alignment using Bio-Edit software. The detected mutations were analyzed using bioinformatics tools, the information regarding the detected SNPs were obtained from National Center for Biological Information (NCBI), as for predicting damaging amino acid substitutions, mutation taster was used in addition to HOPE.

#### Statistical Analysis

Demographic, clinical, and sequencing findings were performed by Microsoft Office Excel, and Statistical Package for Social Science Program 2010 SPSS version 25, which included frequencies, cross-tabulation.

## 4. Result

### 4.1. Demographic Results

Ninety-nine patients diagnosed with idiopathic epilepsy were recruited in this study, Highest age group category was 18 - 40 years accounted for 55% of Patients, followed by less than 18 years group (32%), then 41 - 65 years (11%) while more than 65 years group were only 2% (Table 1).

Regarding gender, females were the majority with 53% and 47% males (Table 2).

Fifty percent of the patients had the first seizure at age less than 5 yrs, 28% of them had it in age between 5 - 10 years 22% had the onset of seizure at age more than10 years (Table 3).

Ninety percent of the patients have no Family history with epilepsy while the rest of them (10%) declared to have a family history (Table 4).

### 4.2. Biochemical Results

Cross-tabulation of calcium among patients revealed calcium level < 8.5 mg/dL was found in 54.4% of the patients followed by 39.4% with normal calcium level (8.5 - 10.5) (Table 5).

Table 1. The age groups in idiopathic epileptic patients.

| Age | No of patients | % | P-value |
|---|---|---|---|
| More than 65 years | 2 | 2.0% | |
| Less than 18 years | 32 | 32.0% | <0.001 |
| 18 - 40 years | 55 | 55.0% | |

Table 2. The gender domination in idiopathic epileptic patients.

| Gender | No of patients | % | P-value |
|---|---|---|---|
| Female | 53 | 53.0% | 0.549 |
| Male | 47 | 47.0% | |

Table 3. The onset of seizure in age groups among idiopathic epileptic patients.

| Onset of seizure | No of patients | % | P-value |
|---|---|---|---|
| Less than 5 years | 50 | 50.0% | |
| More than 10 years | 22 | 22.0% | 0.001 |
| 5 - 10 years | 28 | 28.0% | |

Table 4. The family history among idiopathic epileptic patients.

| Family history | No of patients | % | P-value |
|---|---|---|---|
| No | 90 | 90.0% | <0.001 |
| Yes | 10 | 10.0% | |

### 4.3. Molecular and Sequencing Results

In the current study, the sequencing findings of the SCN1A gene showed that, at chr2:166848853 C>G was detected in (57%) of the samples, while C>T was detected in 14%, both mutations showed splice site effect. Deletion AT was detected at chr2:166848848-49 in 71% of samples. Also C>T on chr2:166848847 was detected (71%) with splice site effect, T>A single base exchange was detected in (14%) on chr2:166848841 with splice site effect, in addition, Deletion A at positions chr2:166848832 and chr2:166848824, were observed in 71% of the samples which appear to have a splice site effect (**Figure 1**).

### 4.4. Bioinformatics Results

Furthermore, SCN1A gene mutations were analyzed using mutation taster program (http://www.mutationtaster.org/) to obtain the type and location of mutations, while Hope database (https://www3.cmbi.umcn.nl/hope/) was used to detect the mutation impact upon the protein. The findings of mutation taster program showed that deletion AT and A created frame shift mutations with amino acid change at positions I1646P and G1652E respectively, **Figures 2-5**. In addition to that, deletion A showed amino acid change at position 1654 from Lysine to Arginine (**Figure 6**, **Figure 7**).

## 5. Discussion

Epilepsy is one of the most frequent neurological disorders; the pooled incidence rate of epilepsy was 61.4 per 100,000 person-years (95% CI 50.7 - 74.4) [14] with a 3% risk of developing epilepsy across all ages [15].

**Table 5.** Calcium levels in idiopathic epileptic patients.

| Calcium (mg/dL) | Frequency | Percentage % |
|---|---|---|
| Less than 8.5 | 54 | 54.4% |
| 8.5 to 10.5 | 39 | 39.4% |
| More than 10.5 | 6 | 6.1% |

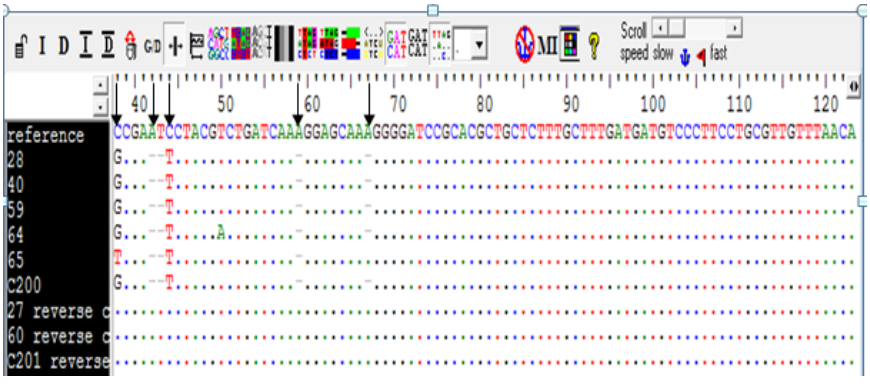
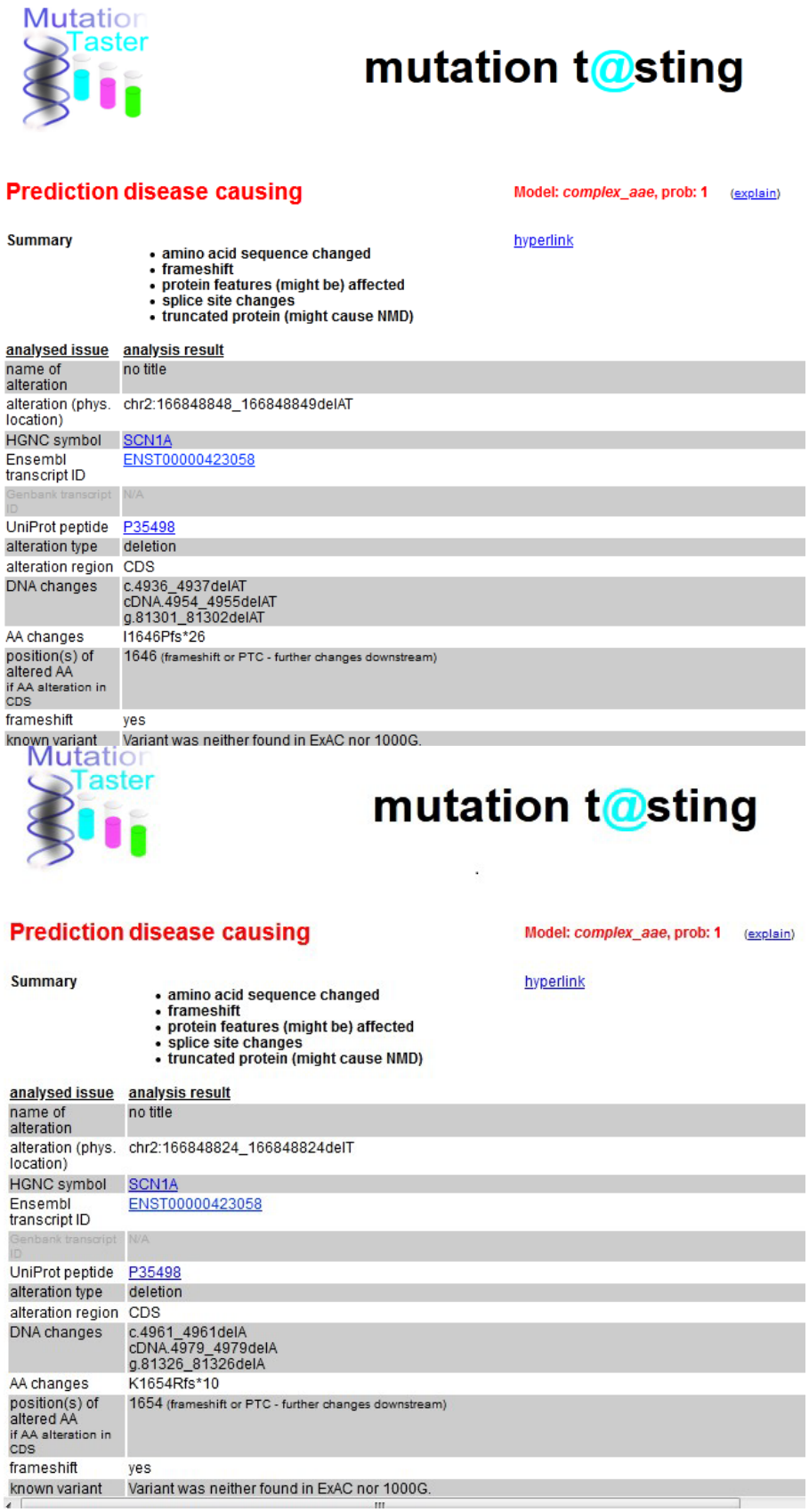



**Figure 1.** The alignment of SCN1A gene sequence obtained from NCBI database with our samples, using BioEdit program. Arrows indicates mutations sites.



mutation t@sting

**Prediction disease causing**

Model: *complex_aae*, prob: 1 (explain)

**Summary**

hyperlink

- amino acid sequence changed
- frameshift
- protein features (might be) affected
- splice site changes
- truncated protein (might cause NMD)

| analysed issue | analysis result |
|---|---|
| name of alteration | no title |
| alteration (phys. location) | chr2:166848848_166848849delAT |
| HGNC symbol | SCN1A |
| Ensembl transcript ID | ENST00000423058 |
| Genbank transcript ID | N/A |
| UniProt peptide | P35498 |
| alteration type | deletion |
| alteration region | CDS |
| DNA changes | c.4936_4937delAT<br>cDNA.4954_4955delAT<br>g.81301_81302delAT |
| AA changes | I1646Pfs*26 |
| position(s) of altered AA if AA alteration in CDS | 1646 (frameshift or PTC - further changes downstream) |
| frameshift | yes |
| known variant | Variant was neither found in ExAC nor 1000G. |

mutation t@sting

**Prediction disease causing**

Model: *complex_aae*, prob: 1 (explain)

**Summary**

hyperlink

- amino acid sequence changed
- frameshift
- protein features (might be) affected
- splice site changes
- truncated protein (might cause NMD)

| analysed issue | analysis result |
|---|---|
| name of alteration | no title |
| alteration (phys. location) | chr2:166848824_166848824delT |
| HGNC symbol | SCN1A |
| Ensembl transcript ID | ENST00000423058 |
| Genbank transcript ID | N/A |
| UniProt peptide | P35498 |
| alteration type | deletion |
| alteration region | CDS |
| DNA changes | c.4961_4961delA<br>cDNA.4979_4979delA<br>g.81326_81326delA |
| AA changes | K1654Rfs*10 |
| position(s) of altered AA if AA alteration in CDS | 1654 (frameshift or PTC - further changes downstream) |
| frameshift | yes |
| known variant | Variant was neither found in ExAC nor 1000G. |

**Figure 2.** Deletion AT mutation at chr2:166848848-166848849 using Mutation taster program.

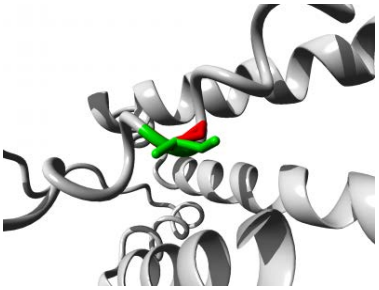


**Figure 3.** The wild and mutant aa residues at position (I1646P) using Hope program (PDB:6AGF). The wild type is in green and the mutant in red color.

Mutation Taster

mutation t@sting

**Prediction disease causing** Model: *complex_aae*, prob: 1 (explain)

**Summary** hyperlink

- **amino acid sequence changed**
- **frameshift**
- **protein features (might be) affected**
- **splice site changes**
- **truncated protein (might cause NMD)**

| analysed issue | analysis result |
|---|---|
| name of alteration | no title |
| alteration (phys. location) | chr2:166848832_166848832delT |
| HGNC symbol | SCN1A |
| Ensembl transcript ID | ENST00000423058 |
| Genbank transcript ID | N/A |
| UniProt peptide | P35498 |
| alteration type | deletion |
| alteration region | CDS |
| DNA changes | c.4953_4953delA<br>cDNA.4971_4971delA<br>g.81318_81318delA |
| AA changes | G1652Efs*12 |
| position(s) of altered AA<br>if AA alteration in CDS | 1652 (frameshift or PTC - further changes downstream) |
| frameshift | yes |
| known variant | Variant was neither found in ExAC nor 1000G. |

**Figure 4.** Deletion A mutation at chr2:166848832-166848832 using Mutation taster program.

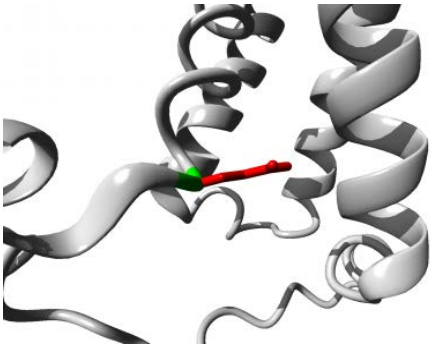


**Figure 5.** The wild and mutant aa residues at position (G1652E) using Hope program (PDB:6AGF). The wild type is in green and the mutant in red color.

mutation t@sting

**Prediction disease causing** Model: *complex_aae*, prob: 1 (explain)

**Summary** hyperlink

- amino acid sequence changed
- frameshift
- protein features (might be) affected
- splice site changes
- truncated protein (might cause NMD)

| analysed issue | analysis result |
|---|---|
| name of alteration | no title |
| alteration (phys. location) | chr2:166848824_166848824delT |
| HGNC symbol | SCN1A |
| Ensembl transcript ID | ENST00000423058 |
| Genbank transcript ID | N/A |
| UniProt peptide | P35498 |
| alteration type | deletion |
| alteration region | CDS |
| DNA changes | c.4961_4961delA<br>cDNA.4979_4979delA<br>g.81326_81326delA |
| AA changes | K1654Rfs*10 |
| position(s) of altered AA<br>if AA alteration in CDS | 1654 (frameshift or PTC - further changes downstream) |
| frameshift | yes |
| known variant | Variant was neither found in ExAC nor 1000G |

**Figure 6.** Deletion A mutation at chr2:166848824-166848824 using Mutation taster program.

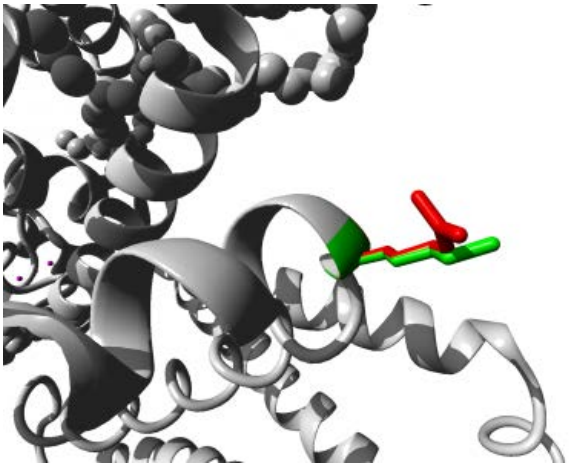


**Figure 7.** The wild and mutant aa residues at position (K1654R) using Hope program (PDB:6AGF). The wild type is in green and the mutant in red color.

The current study was performed on 99 patients with idiopathic epilepsy; it showed that 50% of the participants had onset of the disease in an age less than 5 years old. Though according to Marini Cet [16] there is a shred of increasing

evidence for the existence of IGE beginning beyond the third decade.

Regarding gender, males were less than females 53% females, 47% male. Similar findings were conducted in Asadi study [17] females 57%, 43% were males. Outnumbering of females over males is expected as Studies showed that female sex steroid hormones have repeatedly been shown to affect neuronal excitability [18].

The diagnosis of idiopathic epilepsy starts with a questionnaire establishing the Family History of Epilepsy (FHE) on these patients [19]. In this study, 90% of the patients have no Family history with epilepsy while the rest of them (10%) declared to have a family history. Unlike Babtain FA study results [20] which showed a significant association between idiopathic epilepsy and the presence of FHE.

Calcium level was normal (mean 8.32 mg/dl and SD 1.25), while A prospective case-control study done by Oladipo O [21] showed that calcium levels are lower in children with epilepsy compared to the controls during the seizure-free periods.

Nearly 70% of epileptic patients lack an obvious pathogenetic cause and genetics are believed to play a vital role in its causation [22] 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. Mutation in the SCN1A gene causes defective gating in the sodium channel and thus causes hyperexcitability and seizures [23]. More recent studies have indicated that common variants in the SCN1A gene may be risk factors for common epilepsies like temporal lobe epilepsy and idiopathic/genetic generalized epilepsy (GGE/IGE) [24]. Factors that do not necessarily change the structure of the gene however do affect the progression of transcription and translation is recognized as epigenetic factors [25]. Any perturbation in the transcription, translation, or epigenetic mechanisms can generate defective proteins which lead to diseases. Seizures occur as a result of a complex interplay of altered gene expressions, increased neuronal excitability, and disturbed intrinsic neuronal properties [26] [27]. Defects in epilepsy genes give a critical insight into the pathomechanisms of seizure generation and propagation, which has an impact on the management of the patients [28].

In this study C > T on chr2:166848847 mutation was detected with single base exchange alteration type and splice site effect, similar mutation was reported earlier in NCBI (rs777853016).

Also several deletions were detected on SCN1A gene which is located in chromosome 2, it was found that AT deletion at chr2: 166848848_166848849 changed the amino acid sequence at position 1646, protein features (might be) affected and the splice site had changed. As a result, isoleucine muted into a proline, with the size difference, mutation caused an empty space in the core of the protein. L proline is a GABA-analogue, its accumulation in the cytosol of GABAergic neurons causes competitive inhibition of Glutamic Acid Decarboxylase (GAD) leading to deficient gamma-aminobutyric acid (GABA) production [29],

GABA is the main inhibitory neurotransmitter in the cerebral cortex, it maintains the inhibitory tone that counterbalances neuronal excitation. When this balance is disturbed, seizures may arise [30].

A deletion at chr2: 166848832_166848832 (frame-shift mutation) made a change in the amino acid sequence with glycine mutation into a glutamic Acid at position 1652. The most flexible of all residues is glycine. This flexibility might be required for the protein's function. Mutation of this glycine can eliminate this function.

The wild-type residue charge was neutral, while the mutant residue charge is negative, this can cause repulsion between the mutant residue and neighboring residues. Also, glycine location is on the surface of the protein, alteration of this residue can disturb interactions with other molecules or other parts of the protein. It should be mentioned that the torsion angles for glycine are unusual .only glycine is flexible enough to make these torsion angles, so any mutation will develop an incorrect confirmation which will disturb the local structure [31]. glutamic acid is considered to be a major excitatory neurotransmitter in the vertebrate CNS [32]. Studies conducted in the past few decades are evidence for the role of glutamic acid in epilepsy [33].

A>- was identified at 166848824_166848824, with a frame-shift mutation it caused a change in the amino acid sequence with mutation of a Lysine into an arginine at position 1654. Arginine, also known as l-arginine (symbol Arg or R), is an $\alpha$-amino acid that is used in the biosynthesis of proteins [34]. It is a precursor for the synthesis of Nitric Oxide (NO) [35]. Nitric Oxide (NO) is a short-lived, gaseous signaling molecule that is produced endogenously by a family of enzymes called the Nitric Oxide Synthases (NOS), which catalyze the synthesis of NO from the amino acid arginine [36] it have been pointed out as potential neurotransmitters or retrograde messengers [37] linked to synaptic plasticity [38] and regulation of brain excitability, including the triggering of seizure activity [39] [40]. The involvement of NO in epileptic disorders has been shown in experiments with systemic injection of NOS inhibitors [39] [41].

The study was limited by two factors, first, the sequencing analysis was done to selected samples, this is due to the limited financial source, this research was self funded and no any other institutional or governmental funded given. Secondly, the sample size is not like other published articles in the same field, that is because the majority of the patients refused to be included in the study and we didn't find enough collaboration with the co-patients and the patients.

## 6. Conclusion

A deletion mutation in SCN1A gene (frameshift) can cause epilepsy by changing some amino acids with residues that can affect neuronal stability indirectly.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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