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Sodium voltage-gated channel alpha subunit 9 mutation in epilepsy

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Abstract. **In humans, gene mutations in voltage-gated sodium channels can cause a range of epileptic symptoms, including genetic (generalized) epilepsy with febrile seizures plus (GEFS+) and Dravet syndrome (DS). The SCN9A is a member of the SCN9 gene family that encodes sodium transporter proteins. In the current case report, we delineate a 12-year-old patient who was referred to a pediatric neurology clinic for infantile-onset generalized epileptic seizures and progressive neurodevelopmental delay. Novel heterozygous mutations c.4702A>C (p.Asn1568His) in the SCN9A gene, and c.65G>A (p.Arg22Gln) in the MLC1 gene were detected using targeted next-generation gene sequencing. The replacement of Histidine (His) with Asparagine (Asn) at position 1568 in the topological domain of SCN9A channel protein provides new insights into the impaired excitation and inactivation patterns of sodium channels. The case report adds this new patient with genetic link of SCN9A variants with progressive myoclonic epilepsy and cognitive difficulties.**

Key Words:
SCN9A, Clinical manifestations, Gene variations.

Introduction

Epilepsy is a neurological condition characterized by a long-term predisposition to epileptic seizures; it has social, psychological, and cognitive effects[1]. The economic burden of epilepsy on healthcare systems depends on the diagnosis, the severity of the disease, and the treatment implemented[1]. The causes of epilepsy may include (but are not limited to) acquired defects, autoimmune deficiencies, infections, and genetic mutations, all of which may also influence patients' responses to antiepileptic drugs (AEDs)[2].

The sodium voltage-gated channel alpha subunit 9 (SCN9A) gene belongs to a group of genes that encodes sodium channels; they are also believed to play an important role in epilepsy[3]. A voltage-gated sodium channel is a heteromeric protein composed of one alpha and one or more beta subunits and positively charged sodium atoms (sodium ions). These channels play a key role in a cell's ability to generate and transmit electrical signals[4]. SCN9A mutations have been previously identified in the families of patients diagnosed with autosomal dominant febrile seizures (FS), generalized epilepsy with febrile seizures plus (GEFS+), or sporadic Dravet syndrome (DS)[5]. It has also been suggested[5] that SCN9A mutations may function as a genetic modifier. Furthermore, SCN9A mutations may be triggered by a high fever in patients affected by febrile seizures.

Megalencephalic leukoencephalopathy (MLC1) is a rare childhood-onset leukodystrophy caused by homozygous recessive mutations[6]. MLC1 is a membrane protein that is exclusively expressed in brain astrocytes. It is clinically characterized by macrocephaly, deterioration of motor function with ataxia and spasticity, epileptic seizures, and mental decline[7]. This case report from local population and adds this new patient to the literature of SCN9A gene mutation.

Case Presentation

A 12-year-old boy was referred to the pediatric neurology clinic due to global development delay (GDD) and frequent myoclonic jerks. The onset of myoclonic jerks appeared at the age of 7-years and patient started to experience left hemi-facial myoclonic twitches and hemi-body jerks and involved the upper and lower limbs that cause unbalanced posture while walking. The frequency of seizures varies 10 to 15 times per day and each lasting 1-2 minutes in duration. The treatment was initiated with Sodium valproate: 360 mg

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twice daily (b.i.d.), which reduces the frequency of seizures to none. However, after few months, his condition deteriorated and the incidence of seizures became risen despite regular doses of Sodium valproate. In this situation, parents decided to stop the treatment. The patient was not on any medication, when he was referred to King Fahad Specialist Hospital, Dammam.

He had no significant medical or surgical history, indicating that he was delivered at full term after an uneventful pregnancy. He experienced a smooth perinatal course, and he and his mother were discharged on the second day after his birth in good condition. He had no history of status epilepticus, and he had previously been treated with valproic acid.

He was able to walk but experienced frequent falls due to myoclonic jerks. His fine motor skills were impaired by a tremor, and he ccoul not eat with a spoon or drink from a cup. His speech was only understandable to his father. He had severe learning difficulties and stopped attending school after repeating the third grade three times. His parents were non-consanguineous, and he has seven healthy siblings. There was no family history of epilepsy, developmental delay, or intellectual disabilities.

He was conscious and alert and made minimal eye contact during the physical exam. His head circumference was 50 cm (50th percentile). He did not have neuro-cutaneous stigmata. He experienced a myoclonic jerk during the assessment. His cranial nerves appeared normal, and his motor evaluation was non-focal and unremarkable. His gait was normal apart from some myoclonic jerks that occurred while walking; the physical exam revealed no other remarkable findings and no ataxia.

His metabolic screen including ammonia, lactate, serum amino acid, urine amino acid, urine organic acid, pyruvate, carnitine, acylcarnitine, and fatty acid was normal. His ophthalmology and hearing assessment was normal. A brain magnetic resonance image (MRI) axial flair image showed mild generalized atrophy (Figure 1). He underwent long-term monitoring (LTM) *via* video EEG in October 2017. The LTM was abnormal and revealed generalized slowing of the background, frequent irregular generalized epileptic discharges (Figure 2A), and multiple clinical and electrographic seizures correlating with generalized onset (Figure 2B).

A high throughput sequencing panel of 343 genes was selected by CGC Genetics. Only

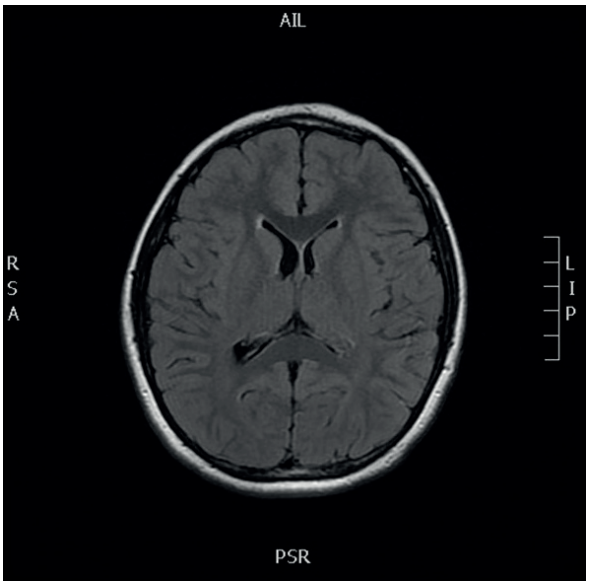

Figure 1. Brain MRI axial flair image showing mild generalized atrophy.

two mutations were detected in the SCN9A and MLC1 genes respectively. One variant of SCN9A was detected in heterozygosity indicating dbSNP (rs755887452). The variation encodes Histidine (His) residue at 1568 instead of Asparagine (Asn) in SCN9A (isoform 3 with missing amino acid residues 648-658).

SCN9A gene (17784 bp) comprised of 27 exons (OMIM 603415.0020) (Figure 3). The encoded sodium channel is composed of 1977 amino acids (225 KDa) organized into 4 domains, each with 6 transmembrane segments[8], and is predominantly expressed in the hippocampus, cerebral cortex, dorsal root ganglion (DRG) neurons, and sympathetic ganglion neurons[9]. SCN9A mutation found in this case found in the highly conserved amino acid residues located in the extracellular region of domain IV of the SCN9A sodium channel alpha subunit is associated with myoclonic epilepsy and cognitive dysfunction (Figure 3). Previously, known variations in the SCN9A include, I62V, I139V, P149Q, S490N, N641Y, K666R leading to febrile seizures (FS), Darvet syndrome (DS), and myoclonic epilepsy with implication in gain-of-function and hyper-excitability of the channel[10]. Other than I139V, all FS, DS, and myoclonic epilepsy-associated missense mutations were predominantly found in the topological motifs of the peptide. In the present study, the detected missense mutation is similarly delineated in the topological domain (Asn1568His). The cellular mechanism by

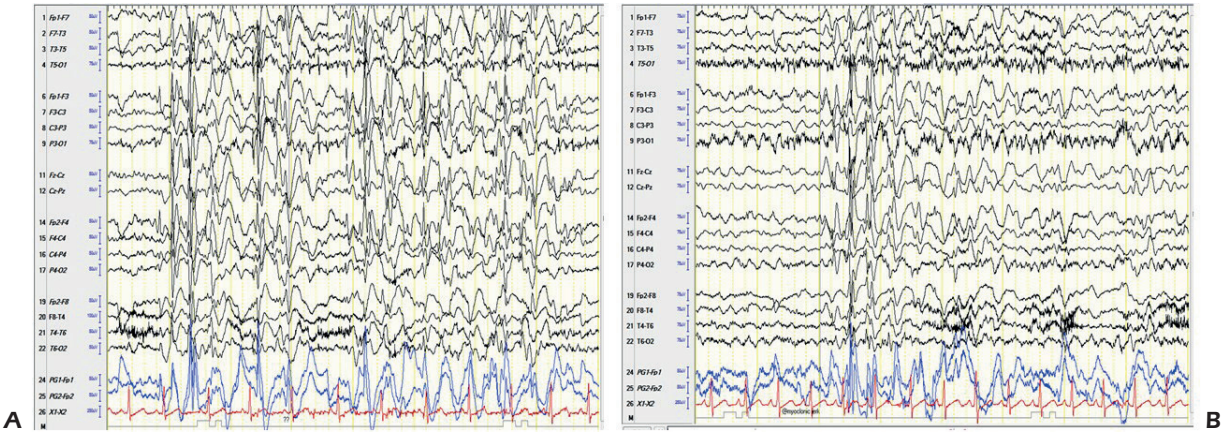

Figure 2. A, EEG data showed a poorly organized background and a generalized irregular burst of 2-4 Hz high amplitude spike, polyspike, and sharp waves intermixed with slow waves. **B,** Image showed myoclonic jerk associated with a burst of generalized epileptic discharges with bifrontal predominance for one second followed by 3-4 Hz slow waves lasting for few seconds.

which these different missense mutations lead to increased seizure susceptibility remains to be elucidated, although one likely explanation is the hyperexcitability of SCN9A. Depending on the specific amino acid change and its location in the protein, there is a potential for altered interactions with binding partners, aberrant post-translational modification, and/or conformational changes. Collectively, these data confirm that SCN9A missense mutations are disease-causing for FS and suggest a role for SCN9A as a modifier of DS. Other SCN9A mutations are associated with different inherited disorders including primary erythermalgia[10,11], paroxysmal extreme pain disorder[12], and channelopathy-associated insensitivity to pain[13], however, none of the variants for FS and DS overlap with the variants conferring these other disorders. Therefore, specific mutations, or a combination thereof, could lead to either enhance or reduce excitability or alter the inactivation of sodium channels (Nav1.7), resulting in the distinct phenotypes conferred by the different mutations.

No additional mutations were detected in other analyzed genes (the epilepsy panel). A second heterozygous variant (c.65G>A (p.Arg22Gln) was detected in the MLC1 gene. The genetic result for the parents was unremarkable.

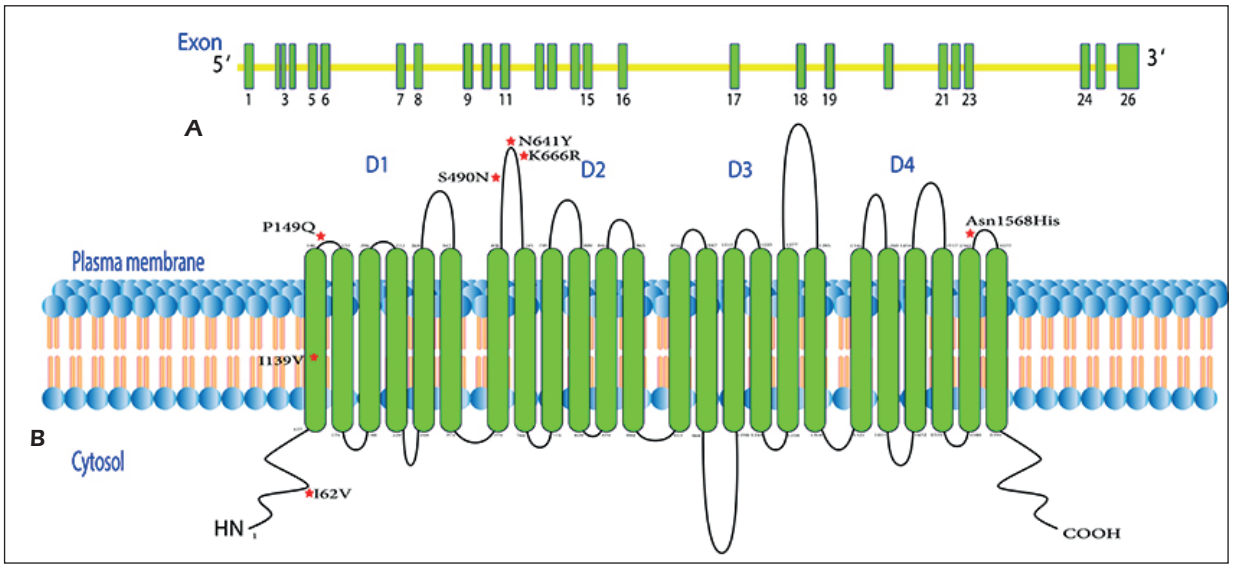

Figure 3. SCN9A mutation found in this case found in the highly conserved amino acid residues located in the extracellular region of domain IV of the SCN9A sodium channel alpha subunit, is associated with myoclonic epilepsy and cognitive dysfunction.

The patient is currently taking levetiracetam (1200 mg orally (b.i.d.); 54 mg/kg/day) and LAMICTAL: 10 mg/daily with some seizure reduction by around 25%, and we are optimizing this drug to reach maximum dose and look at the efficacy.

Discussion

This case study aims to identify rare variants that contribute to familial myoclonic epilepsy and cognitive difficulties. Using high throughput sequencing analysis, we identified c.4702A>C (p.Asn1568His), a variant in the SCN9A gene that was not previously known in the literature.

This specific SCN9A mutation is reported in dbSNP (rs755887452, MAF N/A) and gnomAD (0.0017% described in four heterozygotes)[8]. It is located in a highly conserved residue, and a bioinformatics analysis indicates that the clinical significance of this variant is unknown. In other diseases, like early infantile epileptic encephalopathy 6 (MIM 077208), SCN9A mutations are characterized by myoclonic seizures and delayed psychomotor development.

The SCN9A gene plays a key role in the central nervous system; traditionally, it has been believed to play a particularly important role in the peripheral nervous system. Familial febrile seizures[4], a benign form of epilepsy, are associated with missense SCN9A mutations. It is also associated with DS, which presents with GDD and autistic-like behaviors and is associated with an increased ratio of excitatory to inhibitory neurotransmitters in the brain[14].

The c.65G>A (p.Arg22Gln) variant of the MLC1 gene has been described in patients with leukoencephalopathy[15]. Functional studies conclude that this variant does not affect mRNA or protein expression, but it causes the protein to be trapped in the endoplasmic reticulum, thereby inhibiting its function at the cell membrane. This variant is also reported in the dbSNP (rs184241759, MAF:0.2%) and the gnomAD (0.045%). This mutation in the MLC1 gene causes megalencephalic leukoencephalopathy with subcortical cysts 1 (MIM 604004). It is autosomal recessive, so the presence of this variant alone is not sufficient to cause disease, although it is pathogenic. No additional mutations were detected in this gene. If the patient's phenotype was considered compatible, testing for deletions/duplications in this gene by MLPA could be considered.

The importance of the location of the mutation of single amino acid for the channel function and described two novel non-truncating mutations in SCN9A for channelopathy-associated insensitivity to pain[14]. Another study[14] showed the SCN9A variant inhibits the membrane trafficking function for the chaperone protein that correctly folds Nav1.7.

The genes co-expressed with SCN9A in the brain indicate that behavior might be a prominent component of the phenotype associated with SCN9A[8]. The behavior of our patient with GDD suggests that patients with myoclonic jerks and deregulated behaviors might be enriched for SCN9A variants. Deregulated behaviors could also indicate a phenotype that is suitable for genetic studies, and it might be useful to examine this phenotype in genetic studies of myoclonic epilepsy[15,16].

Conclusions

Preventive programs that include prenatal screening and genetic counseling are important in countries like Saudi Arabia, where consanguineous marriages are common and the expression of autosomal recessive genetic disorders may lead to severe epilepsy. The current case study reports novel heterozygous mutation c.4702A>C (p.Asn1568His) in the SCN9A gene of the patient. The detection of a unique missense mutation in the SCN9A genes may lead to altering the behavior of sodium channels in terms of impaired excitability and/or altered inactivation patterns resulting in the specific phenotypes in the distinct type of channelopathies.

Conflict of Interests

Authors have no conflict of interests and the work was not supported or funded by any drug company.

Consent for Publication

Not applicable.

Ethics Approval and Consent to Participate

The protocol of research was reviewed and approved by the Ethics and Research Committee of the King Fahad Specialist Hospital Dammam.

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