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CASE REPORT

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A Novel *SLC9A6* Mutation Associated with Christianson Syndrome in a Non-Consanguineous Iranian Family: A Case Report

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Abstract

Background: Christianson syndrome is a neurological disorder characterized by recurrent seizures resulting from abnormal brain neuron discharge. Primarily emerging in childhood, Christianson syndrome is marked by a complex clinical presentation intertwined with social and learning disabilities. Moreover, it is often associated with severe epilepsy, encompassing various debilitating neurological conditions marked by frequent seizures and developmental challenges.

Case Presentation: A 2-year-old Iranian boy presented with developmental delays and tonic-clonic seizures. Physical examination revealed microcephaly, motor delays, and other neurological abnormalities. Genetic testing identified a novel hemizygous missense mutation, c.577G>A; p.Gly193Arg, in the *SLC9A6* gene. This mutation likely contributes to Christianson syndrome, characterized by intellectual disability, epilepsy, and other neurological impairments. Further validation confirmed the mutation's presence, highlighting its significance in the patient's clinical presentation.

Conclusion: The c.577G>A; p.Gly193Arg mutation in the *SLC9A6* gene sheds light on the pathogenesis of Christianson syndrome by disrupting the normal function of the SLC9A6 protein crucial for neuronal development. This discovery underscores the significance of *SLC9A6* in maintaining cellular homeostasis and highlights its role in neurological disorders. Further exploration of this mutation's effects may lead to targeted therapeutic strategies for managing the debilitating symptoms of Christianson syndrome.(**International Journal of Biomedicine. 2024;14(3):536-540.)**

Keywords: Christianson syndrome • *SLC9A6* gene • mutation

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Introduction

Christianson syndrome (CS) arises from the sudden abnormal discharge of brain neurons, leading to recurrent seizures attributed to hypersynchronous activity.[1] Typically manifesting in childhood, CS presents a multifaceted clinical profile intertwined with social and learning disabilities. Additionally, severe epilepsy encompasses a cluster of debilitating neurological conditions characterized by frequent epileptic seizures coupled with developmental delay or regression.[2] Each epilepsy subtype exhibits distinct clinical features and underlying pathophysiological or genetic mechanisms. Unveiling the root cause of epilepsy or a specific electroclinical syndrome is paramount for comprehending

its natural progression and determining the most effective treatment strategies.[3]

To date, a plethora of genes, among them Na^+/H^+ exchanger protein 6 (*NHE6*, also referred to as *SLC9A6*), have been implicated in CS.[2] *NHE6* serves a pivotal role in both excitatory presynaptic and postsynaptic functions.[4] Moreover, its robust expression in the brain underscores its involvement in intracellular vesicle targeting and synaptic vesicle recycling. Consequently, mutations resulting in the loss of SLC9A6 function may impede neuronal development.[5] Furthermore, *SLC9A6* has been identified as a pathogenic gene,[6] with its pathogenic variants giving rise to an Angelman-like syndrome (ASlike, known as CS).[7] This syndrome is typified by epilepsy, intellectual disability, microcephaly, ophthalmoplegia, craniofacial dysmorphism, and progressive cerebellar atrophy.[8]

Childhood epilepsy, particularly seizures, unfolds within the dynamic landscape of brain development and exhibits evolving characteristics over time. In children, CS poses a spectrum of treatment complexities specific to this developmental stage. Hence, it becomes imperative to differentiate between mutations underlying childhood epilepsy or hereditary childhood epilepsy. Tailoring effective therapies for various epilepsy forms becomes paramount, addressing the unique needs of each child based on their seizure type. Exome-sequencing emerges as an indispensable asset in unraveling the etiology of diseases, especially those stemming from rare gene mutations.[9-12]

In our study, we have uncovered a novel *SLC9A6* missense mutation, c.577G>A, within an Iranian patient exhibiting tonic-clonic seizures and generalized developmental delay, employing Exome-sequencing techniques. Additionally, we have synthesized previously reported pathogenic variants, thereby broadening the spectrum of *SLC9A6* mutations.

Case Presentation

A 2-year-old boy from a non-consanguineous Iranian family (Figure 1A) presented with a myriad of developmental concerns, which had been a source of growing apprehension for his parents. Despite efforts to support his growth and development, they observed persistent delays in achieving developmental milestones, alongside concerning behaviors. These challenges prompted the family to seek consultation with a psychologist for further evaluation. Upon assessment, the psychologist noted features consistent with moderate intellectual disability, reflecting the child's struggles with cognitive and adaptive functioning.

During the consultation, the mother provided valuable insight into the family's medical history, disclosing that her brother had experienced similar developmental challenges. This revelation raised suspicion of a potential genetic component underlying the child's condition. Recognizing the significance of this familial pattern, the healthcare team decided to pursue genetic testing to elucidate the precise etiology of the child's developmental issues. This decision was motivated by a desire to not only confirm the diagnosis but also to provide tailored management and support for the child and his family as they navigated the complexities of his condition.

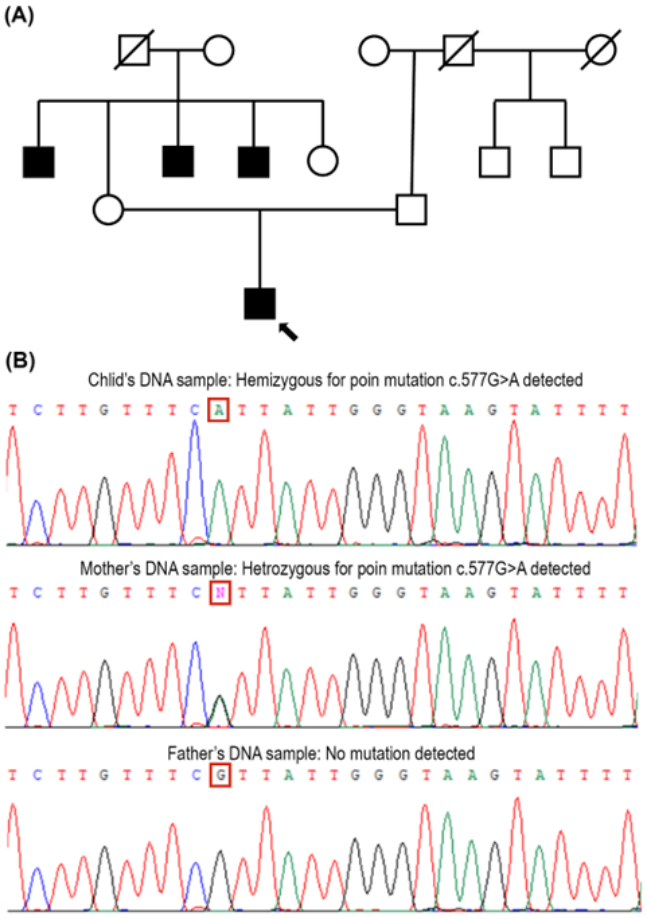

Figure 1. *Family pedigree and genetic analysis: (A) The pedigree showcases male individuals with squares and females with circles. Affected members, highlighted by symbols, include the proband denoted by an arrow. (B) Sequence chromatograms reveal a novel hemizygous mutation c.577G>A; p.Gly193Arg in SLC9A6 within the proband, with the mother displaying a heterozygous state for the mutation, while the father's sequence analysis indicates a normal genotype.*

The patient, born at term with a birth weight of 3200 g, experienced jaundice postnatally but did not require phototherapy. He followed a typical feeding schedule, transitioning from exclusive breastfeeding to supplementary foods appropriately. However, developmental milestones were notably delayed; he achieved rolling over at 5 months, head control at 6 months, and sitting at 9 months. By age 2, he could crawl and stand with support. Despite these advancements, tonic-clonic seizures emerged at 18 months, manifesting multiple times daily.

Physical examination unveiled microcephaly (head circumference: 43 cm, -3SD), motor delays, distal muscle weakness, micrognathia, tonic-clonic seizures, slender fingers, adduction of the thumbs, and sporadic episodes of unprovoked laughter. Additionally, he exhibited difficulties with swallowing and speech. Gesell Development Schedules (GDS) were employed to assess his developmental status, revealing delays across various domains, including adaptation, large and fine motor movements, personal-social development (mild), and language (moderate).

The patient received a diagnosis of tonic-clonic seizures and generalized developmental delay, prompting a

recommendation for rehabilitation training. Sleep EEG results depicted a background activity of 3.5–8 Hz diffuse waves, with spike-slow wave discharges in frontal pole, frontal, anterior temporal, and frontal midline point areas. However, brain magnetic resonance imaging (MRI), brain-stem auditory evoked potential, and visual evoked potential yielded no abnormal findings, further complicating the diagnostic journey.

First, genomic DNA was isolated from blood leukocyte samples using standard salting-out methods. The concentration of DNA samples was determined utilizing a NanoDrop 1000 spectrophotometer. These samples were then either stored at -20º C or immediately subjected to amplification.

Following this, the DNA underwent library production and sequencing capture. A custom Human capture array was employed, designed to encompass all coding regions and intron/exon boundaries of target genes implicated in intellectual disability pathogenesis. This was achieved through exome-sequencing (Macrogen, based in Seoul, South Korea).

Subsequently, sequence alignment of reads was executed against the reference human genome build hg19 from UCSC, with annotations derived from datasets and tools. Frequent variants (frequency >1%) and synonymous substitutions were filtered out using public databases like the 1000 Genome Project (http://www.internationalgenome.org/), dbSNP, and gnomAd (https://gnomad.broadinstitute.org/).

To validate novel variants identified by exome-sequencing, Sanger sequencing was conducted in both the patient and their parents. Specific primers were employed for amplification of target gene sites, referencing the human genome sequences from GenBank in NCBI. PCR products were sequenced directly on an automated genetic analyzer (ABI 3100; Applied Biosystems). Additionally, Sanger sequencing was performed to confirm segregation within the family for the identified candidate variant.

The investigation into the genetic underpinnings of intellectual disability unveiled a significant finding: a previously undocumented mutation within the *SLC9A6* gene in the affected individual. This newly identified mutation, characterized as a hemizygous missense mutation c.577G>A, is situated in exon 6 (NM_001379110.1) of the *SLC9A6* gene. The consequence of this mutation is the replacement of Glycine with Arginine at codon 193 (p.Gly193Arg). The mutation identified here, c.577G>A; p.Gly193Arg, likely contributes to the pathogenesis of Christianson Syndrome by disrupting the normal structure function of the SLC9A6 protein.

Analysis of the DNA sequences of other genes within the familial cohort did not reveal any variants associated with disease causation. Subsequently, the specific region harboring the novel mutation was amplified and subjected to sequencing via polymerase chain reaction (PCR). The presence of the identified novel mutation was then validated through Sanger sequencing, as depicted in Figure 1B.

Discussion

In the realm of neurodevelopmental disorders, CS stands as a rare yet increasingly recognized condition, marked by X-linked intellectual disability. Recent research has shed light on the intricate mechanisms underlying this syndrome, particularly focusing on the role of *NHE6*, an ion transporter pivotal for recycling endosomal pH homeostasis and trafficking.[13] NHE6's significant expression within the central nervous system (CNS) underscores its importance in neural function. Its dysregulation, often stemming from mutations in the *SLC9A6* gene, is implicated in the complex and varied neural manifestations observed in CS. Understanding the specific receptors dependent on *NHE6* has become paramount in unraveling the underlying neuronal dysfunction characteristic of CS. One such consequence of NHE6 malfunction is the disruption of endosomal acidification, impacting the transport of vesicles containing AMPA receptors to and from the postsynaptic membrane. This insight underscores the intricate interplay between molecular mechanisms and synaptic function in CS pathology.[14] Furthermore, the intricate signaling pathways involving Brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TrkB) have emerged as critical players in neurodevelopment. Barford et al.[15] highlight the indispensability of BDNF/TrkB signaling in dendrite development across various neuronal populations in the CNS. However, in CS, the accelerated degradation of TrkB, compounded by endosomal over-acidification, disrupts this crucial signaling cascade, ultimately leading to aberrant axonal and dendritic growth.[16] The integration of these findings not only enhances our understanding of the pathophysiology of CS but also offers potential avenues for therapeutic intervention, emphasizing the importance of targeted approaches aimed at mitigating the underlying molecular perturbations associated with this complex disorder.

In neurodevelopment and growth, microtubule-associated proteins (MAPs) emerge as pivotal orchestrators, shaping the intricate architecture of the brain.[17] Among these, the Tau protein stands out as the principal MAP within mature neurons, playing a crucial role in their function and structure.[18] Throughout the dynamic process of brain development, the spatiotemporal expression of different tau isoforms exhibits characteristic patterns, reflecting the intricate regulation of tau dynamics during neural maturation.[19] This nuanced regulation suggests the fundamental importance of tau isoforms in the formation and refinement of the brain's intricate neural circuits.[20] Recent research has unveiled a potential interplay between tau deposition and the mutant SLC9A6 protein, shedding light on a novel aspect of neurodegenerative processes.[21] Through meticulous genomic analyses, researchers have uncovered a significant correlation between decreased SLC9A6 expression and elevated tau deposition, underscoring the intricate molecular mechanisms underlying neurodegenerative pathology. The aggregation of tau subtypes into the filamentous content of neurons represents a hallmark pathological event in several neurodegenerative diseases, characterized by the aberrant hyperphosphorylation of tau proteins. Notably, the identification of the c.577G>A; p.Gly193Arg pathogenic variant within the *SLC9A6* gene adds another layer of complexity, disrupting the normal structure and function of the NHE6 protein. This genetic perturbation, leading to the substitution of Glycine with Arginine at codon 193, has profound implications for tau protein stability, potentially exacerbating the pathological

cascade observed in neurodegenerative disorders. Furthermore, the inability of the mutant *SLC9A6* to compensate in affected tissues raises intriguing possibilities regarding the etiology of associated clinical manifestations, such as epilepsy and developmental delays.

The detected mutation c.577G>A; p.Gly193Arg in the SLC9A6 gene is likely to significantly impact the structure and function of the corresponding SLC9A6 protein, potentially leading to the manifestation of CS. This pathogenic variant results in a substitution of glycine to arginine at codon 193, which is predicted to disrupt the normal conformation and functionality of the NHE6 protein. As *SLC9A6* encodes the NHE6 protein, which plays a critical role in regulating pH homeostasis within cellular compartments, its malfunction due to the identified mutation can disrupt various cellular processes essential for neuronal function and development. CS, characterized by intellectual disability, epilepsy, microcephaly, and severe speech impairment, is often associated with mutations in the *SLC9A6* gene. The identified mutation likely contributes to the clinical phenotype of CS by perturbing neuronal homeostasis and function, ultimately underscoring the importance of SLC9A6 in normal neurodevelopment and its implications in neurological disorders.

The mutation's impact on SLC9A6 function underscores its role in the pathogenesis of CS, shedding light on the molecular mechanisms underlying this debilitating condition. Further research into the precise effects of this mutation on protein structure and function could provide valuable insights into potential therapeutic interventions for CS.

In conclusion, the detected mutation c.577G>A; p.Gly193Arg in the *SLC9A6* gene represents a significant finding in our understanding of CS and its underlying molecular mechanisms. This mutation is predicted to profoundly affect the structure and function of the SLC9A6 protein, crucial for maintaining cellular pH homeostasis. Given the critical role of *SLC9A6* in neuronal function and development, its malfunction due to the identified mutation contributes to the clinical phenotype of CS, characterized by intellectual disability, epilepsy, and other neurological impairments. This discovery underscores the importance of *SLC9A6* in normal neurodevelopment and highlights its implications in neurological disorders. Further research into the precise effects of this mutation could pave the way for targeted therapeutic interventions aimed at ameliorating the debilitating symptoms of CS.

Competing Interests

The authors declare that they have no competing interests.

Ethical Considerations

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from the family members for this publication.

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