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Journal of Autism and Epilepsy

Short Communication

SLC6A1 Gene Mutation Caused Epilepsy with Myoclonus-Atonic Seizures: A Case Report Clinical Characteristics and Genetic Analysis

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Submitted: 23 September 2022

Accepted: 17 October 2022

Published: 19 October 2022

ISSN: 2641-7774

Keywords

- Epilepsy with myoclonic-atonic seizure; SLC6A1 gene; Developmental delay; Levetiracetam

Abstract

Objective: To analyze the clinical and genetic characteristics of a child with epilepsy and myoclonus-atonic seizures caused by SLC6A1 gene variation.

Methods: The clinical and electroencephalogram characteristics of a male child with epilepsy and myoclonus-atonic seizures were analyzed. Peripheral blood was collected to extract DNA for medical whole exon gene sequencing. The proband underwent high-throughput sequencing, and the parents underwent Sanger verification to identify gene mutation sites and analyze the relationship between genotype and phenotype.

Results: Male children, before the onset of mental language development lag behind, 1,2 months hot convulsions, 2 years 9 months in seizures, seizure form including the general stiffness matrix cramps seizure, myoclonic seizures and myoclonus loss of tension, and accompanied by inattention, hyperactivity, giving left b raschig 50 mg/kg/d treatment, nearly 8 months without seizures. The results of genetic testing showed that the SLC6A1 gene c.263T>C (p.L88p) heterozygous missense mutation was a spontaneous mutation, which has not been reported in the literature.

Conclusion: SLC6A1 gene mutation is one of the causes of epilepsy with myoclonic dystonic seizures and neurological abnormalities. The detection of new variants enriched the variation spectrum of SLC6A1 gene.

INTRODUCTION

Epilepsy with myoclonic atonic seizure (EMAS) was formerly known as myoclonic-astatic epilepsy. First proposed by German doctor Hermann Doose et al. [1] in 1970, it is a pediatric comprehensive epilepsy syndrome characterized by multiple seizure types, accounting for about 1%-2% of children with epilepsy [2]. The age of onset is from 7 months to 6 years (usually 1-5 years), and the ratio of male to female is about 2:1. EMAS may have a multifactorial genetic component, and about one-third of cases have a positive family history of variable types of seizures [3]. The electroclinical manifestations of EMAS are broad and the prognosis is diverse, ranging from mild cases to epileptic encephalopathy. Therefore, early diagnosis and effective treatment may affect the long-term prognosis of EMAS. This report reports the clinical and genetic features of a child with EMAS caused by a spontaneous mutation of SLC6A1 gene, hoping to improve pediatricians' understanding of EMAS and strengthen the identification of feV-related epilepsy for early diagnosis and treatment.

MATERIALS AND METHODS

General information

The clinical data of a 2 years and 9 months old boy who visited the hospital in April 2021 due to intermittent fever for 15 days and 6 convulsions in 1 day were collected. G3P2 was born at 40 weeks of gestation, with a birth weight of 3300g. The birth history was normal, and the parents were not consanguineous. His father had a history of febrile seizures when he was young. His mother and 11-year-old sister had no history of convulsions. Their parents and sister grew and developed normally. In order to clarify the etiology, medical whole exome testing was completed with the approval of the hospital's medical ethics committee and the informed consent of the patient's parents.

Methods

Exome capture sequencing: EDTA anticoagulated peripheral Blood (2mL) was collected from the children and their parents, and 3-5 μg genomic DNA was extracted according to the steps of the Blood DNA Mini Kit (Simgen Company, China), and the amplification was interrupted. The genes related to epilepsy with psychomotor retardation (SLC6A1, PCDH19, SETD1A, SLC2A1, SLC25A10, GOT2, TBC1D24, KCNQ5, PIGU, RTTN, SCN1A, SCN1B, SCN2A, CHD2, SYNGAP1 and STX) were established 1B et al.). The above target genes were captured by liquid phase capture kit, and then high-throughput sequencing was performed by a new generation sequencer IlluminaHiSeq2000 (Illumina Company,

USA). The average depth of sequencing was not less than 200×, and the data were analyzed by gene sequence bioinformatics to find out the pathogenic genes.

Sanger sequencing verification: Primers were designed for the candidate mutation sites, and PCR amplification was performed to verify the mutation, short fragment deletion or insertion of the positive site, and to determine the mutation of the site. The first generation verification primer information of the proband and his parents was chr3:11059553, the forward primer sequence CATATGGCTTTCCTTGGGC, and the reverse primer sequence AACATAGGAGCCAGCTTCCA.

4.2.3. Analysis of sequencing results: The annotation range of raw NGS data included variations within each exon and its upstream and downstream 10bp regions. The types include missense, synonymous, nonsense, frameshift, integral, splice variant, etc. The quality control of variant data was carried out, and the sequencing coverage depth less than 20X was marked as low-quality variants. Internal databases, dbSNP, ESP6500, ExAC and other population databases were searched, and single nucleotide polymorphisms and low-frequency benign variants were annotated. Predictive software was used to predict the conservatism, pathogenicity and harmfulness of the variation. HGMD, PubMed, Clin Var and other databases were searched to retrieve relevant literature, and the pathogenicity of variants was evaluated according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

RESULTS

Clinical data

The boy, aged 2 years and 9 months, weighed 12.5kg and had a head circumference of 48cm. He raised his head at 4 months, sat unaided at 8 months, asked for help at 18 months, and walked unaided at 15 months. Febrile seizure occurred once at the age of 1 year and 2 months, with body temperature of 38.2°C during convulsion, manifested as generalized tonic-clonic seizures (GTCS), which lasted for 2 minutes and relieved. No abnormalities were found in cranial computed tomography (CT) and electroencephalogram (EEG) examinations. At the age of two and a half years, she suddenly fell without fever while standing and playing for several times, and climbed up by herself in 3-4 seconds. She was not treated. In April 2021, he was admitted to the hospital due to 6 seizures with or without fever within 1 day. The first episode was GTCS with a fever of 39°C for about 15 minutes, which was relieved after intramuscular injection of midazolam in the emergency department of our hospital. After admission, intermittent convulsions manifested as myoclonic or myoclonic atonic seizures for 5 times, low fever or no fever, which lasted for 3-5 seconds and resolved spontaneously. Physical examination: Consciousness, mental reaction, no special face, mild cognitive language, motor development basic normal, no coffee, milk, splash body skin superficial lymph nodes swollen untouched, neck soft passivity, pharyngeal congestion, breathing pattern, double lung breath sounds clear, soft, liver and spleen untouched swelling, tenderness, rebound tenderness and muscle tension, did not hit a bag piece, normal bowel sounds. The limbs moved freely, the muscle strength and muscle tone were normal, the meningeal irritation sign and the pyramidal tract sign were negative. Laboratory tests showed normal levels of blood ammonia, lactic acid, blood glucose, liver and kidney function, electrolytes, and blood and urine metabolic screening [Zhengzhou Jinyu Medical Laboratory Co., LTD.]. Examination of the cerebrospinal fluid was normal. Magnetic resonance imaging of the head was normal (Figure 1). Video electroencephalogram showed mixed 6-8Hz low-medium amplitude activity in the bilateral occipital region, a large number of generalized 3-4Hz low-high amplitude slow wave bursts during wakeup and sleep for 2-several seconds, and a wide range of 2Hz high-extremely high amplitude spike slow wave bursts during sleep for 4-5 seconds. Neuropsychological development screening assessment of children aged 0-6 years: mental age 24.7 months, DQ 75. A variety of seizure forms including myoclonic atonic seizures, GTCS, myoclonic seizures, status epilepticus, etc., accompanied by mental and language development delay, EMAS was considered clinically. Levetiracetam 40mg/kg/d was given as antiepileptic treatment. At the beginning of treatment, the patient fell and had myoclonic seizures twice, both of which were accompanied by fever. Electroencephalogram showed extensive slow waves without abnormal discharge. The child had delayed intelligence and language development accompanied by attention deficit and hyperactivity. At present, he has been treated in our hospital for half a year, but the improvement is not obvious.

Gene variant analysis

The patient had a heterozygous missense mutation in the SLC6A1 gene (Figure 2): a change from thymidine T to cytosine C at nucleotide 263 (c.263T>C), resulting in a change from leucine to proline at amino acid 88 (p.L88p) (Figure 3). Sanger sequencing confirmed that the parents had no variation in this gene locus. This missense mutation has not been included in the 1000 Genomes, ESP6500 and dbsNP databases, and the mutation frequency in the normal population database is low frequency variation, which can provide evidence of PM2 according to ACMG guidelines. According to the bioinformatics protein function comprehensive prediction software REVEL, SIFT, MutationTaster, and GERP+, the prediction results were all harmful. No association of this locus has been reported in the literature database. This variant was rated as suspected pathogenic according to ACMG guidelines.

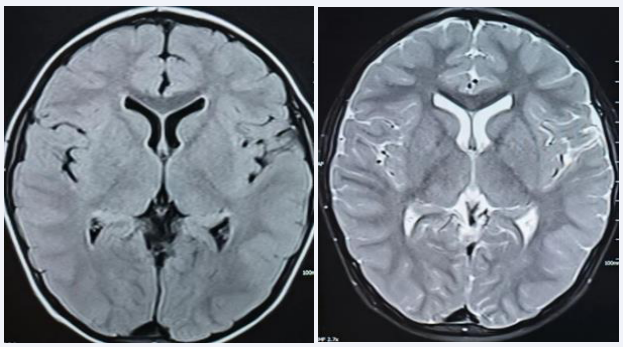

Figure 1 T1(left) and T2(right) weighted images of unenhanced cross-sectional MRI of the child's head.

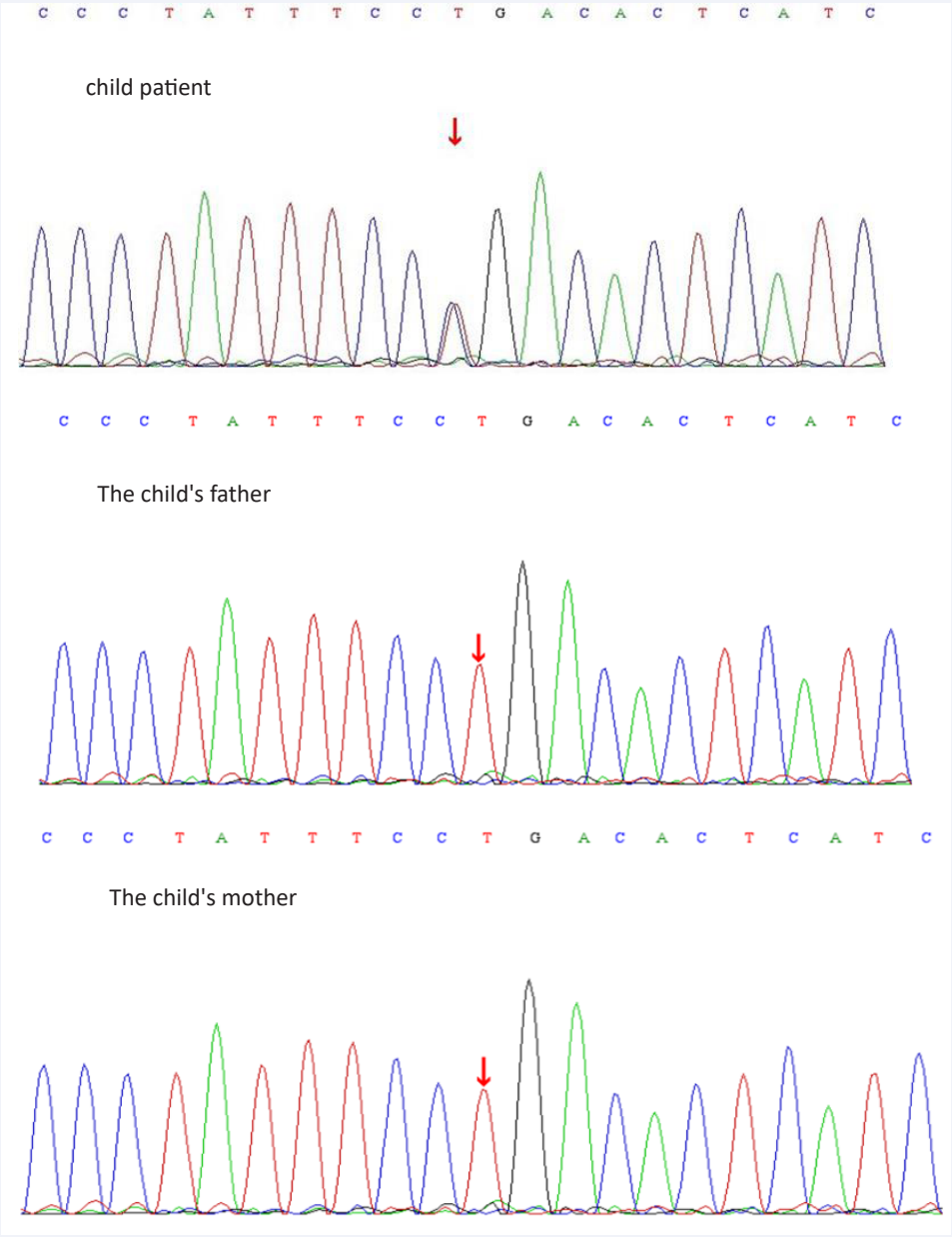

Figure 2 Sanger sequencing results of SLC6A1 gene c.263T>C locus in the child and her parents.

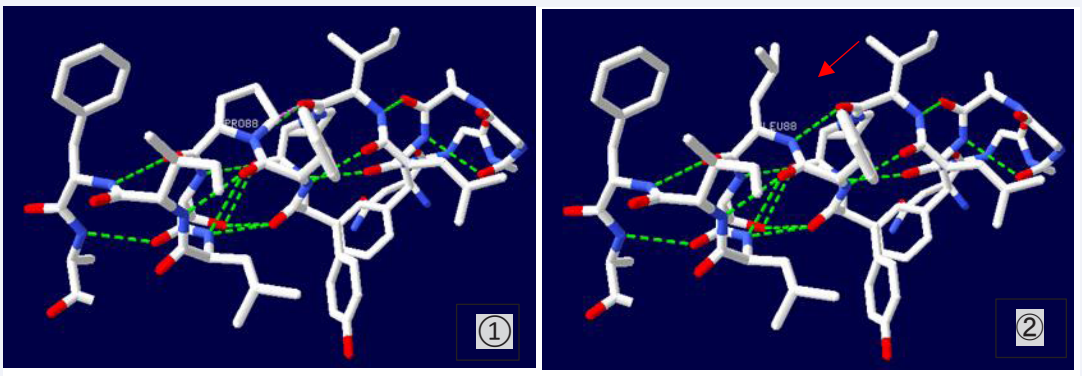

Figure 3 Three-dimensional structure of p. L88p protein ① Wild type; (2) variant.

DISCUSSION

The SLC6A1 gene is localized on chromosome 3p25.3 [4], contains 16 exons, is about 46.5 KB in length, and is widely expressed in human and other animal brains. Gamma-aminobutyric acid (GABA) is an important inhibitory neurotransmitter in the mammalian brain, which prevents excessive excitation of neurons in the brain by regulating inhibitory synaptic transmission [5]. The gamma-aminobutyric acid transporter GAT1, an essential isoform of voltage-dependent GABA transport, is encoded by the SLC6A1 gene [6] and is essential for reuptake of GABA from synapses and clearance of GABA from extracellular space [7,8]. SLC6A1 gene variants lead to decreased GABA transporter activity. Crossman et al. [9] demonstrated that injection of the GABA antagonist bicuculline into the lateral putamen of monkeys caused myoclonic seizures in the contralateral limb. Genetic factors are believed to play a major role in the pathogenesis of EMAS. Genes related to EMAS have been reported, including SCN1A, SCN1B, GABRG2, SLC2A1, SLC6A1, STX1B, SYNGAP1 and micro duplication of 4q21.22-q21.23 [10-12]. The types of variation include missense variation, nonsense variation, frameshift variation, splicing variation and chromosomal microdeletion, among which missense variation is the most common.

In 2015 Johannesen et al. [13], reported that up to 4% of patients with myoclonic atonic epilepsy have de novo SLC6A1 variants. In the same year, Carvill et al. [14], found that among 75 EMAS patients, 2 patients with SLC6A1 mutation were mother and child, and their clinical manifestations were similar, indicating that SLC6A1 mutation may be specific for EMAS. This patient had a heterozygous missense mutation c.263T>C (p.L88P) in the SLC6A1 gene, which was a spontaneous mutation and was evaluated as a suspected pathogenic mutation, which had not been reported previously. Carvill et al. [14], identified SLC6A1 as the cause of neurodevelopmental disorders through two independent whole-exome sequencing studies, which showed de novo mutations in two individuals with intellectual disability and autism. In 2018, Johannesen et al. [13], studied 34 SLC6A1-positive patients and found that most patients had language delay and mild to moderate intellectual disability (ID) before seizures, and a small number of patients had intellectual disability without seizures. SLC6A1 mutations cause a wider range of phenotypes, ranging from normal development to varying degrees of language and motor delay to epileptic encephalopathy. In addition to myoclonic atonic seizures, absence seizures and atonic seizures are more common. This example exists before the onset of epilepsy in children with cognitive language development lag behind, accompanied by inattention, hyperactivity, can communicate with family but language is poorer, backward developmental quotient, basic normal motor development, performance for myoclonus tension seizure, GTCS, myoclonic seizures and hot convulsions persistent state, and other forms, is not evidence of absence seizure, It is basically consistent with foreign reports.

In 2019, Angione et al. [15], analyzed 77 patients with EMAS, 42% of them had a family history of childhood epilepsy, another 19% had a family history of febrile seizures and adult epilepsy, and 5% had relatives without a family history of epilepsy but with other neurodevelopmental abnormalities, including developmental delay, learning disabilities, and autism. The father had a history of febrile seizures when he was a child, but the genotype and phenotype were normal. No information about seizures and neurodevelopmental abnormalities in other relatives was provided. The diversity of clinical phenotypes between EMAS patients and their family members suggests that inheritance may be multifactorial or polygenic. Most of the reported cases are sporadic. Although EMAS is widely accepted to have a strong genetic component, the positive rate of gene diagnosis is still very low, probably because some genes that are now considered to be related to EMAS are not included in the common epilepsy sequence, or have been added recently, and the exact pathogenic genes need to be further studied.

In 2011, Trivisano et al. [16], analyzed 18 patients with EMAS, 88.9% of them were boys, and all of them had myoclonic atonic seizures. Seizures were relieved in 16 patients within 42 months of onset, and the time of seizure cessation was not related to cognitive results. In more than two thirds of cases, febrile and afebrinal GTCS are the initial types and are also seen during the course of the disease. This boy had a history of febrile seizures before the onset, and the first onset was febrile induced GTCS. At the age of 2 years and 9 months, he developed seizures, and GTCS with or without fever was reported, which was consistent with foreign reports. Caraballo et al. [17], analyzed the electroclinical characteristics of 69 children with EMAS in 2013, and found that almost all patients had normal brain imaging and cerebrospinal fluid examination, and interictal EEG showed 2-5Hz generalized spike, multi-spike and wavy discharges. In 2007, Kilaru et al. [18], found that almost all patients with EMAS had complete remission of seizures within 3.5 years, but their cognitive development was uncertain. Studies have found that patients with EMAS caused by SLC6A1 gene variants have seizure relief after treatment with sodium valproate or drugs such as levetiracetam or zonisamide, and some patients have partial response to lamotrigine and sodium valproate. This example with cranial MRI, cerebrospinal fluid examination is normal, attack phase of sleep eeg in wake periods between generalized slow-wave, from small amounts of 2 hz generalized spine slow wave hair, after left b raschig treatment 10 months with no attack, slow review eeg background activities, no abnormal discharge, no cognitive language, with the foreign reports are basically identical. Unlike most epileptic encephalopathy, the prognosis of EMAS is variable, ranging from mild cases to epileptic encephalopathy and from normal cognition to severe intellectual disability, with a favorable outcome most likely if the seizures are resolved without persistent abnormalities on EEG.

There is a lack of consensus on the diagnostic criteria of EMAS, and there is significant variability in clinical practice [19]. Dravet syndrome, West syndrome and Lennox-Gastaut syndrome should be excluded for clinical diagnosis. The history of febrile status epilepticus is most consistent with Dravet syndrome, but febrile seizures can precede EMAS [17]. The child presented with status febrile seizures as the first symptom, and the brain MRI and cerebrospinal fluid examination were normal. There was a history of febrile seizures in the past, and the electroencephalogram was normal at the same time, which was easily misdiagnosed as complex febrile seizures. Family members provided video data of

myoclonic seizures with falls. Combined with age characteristics, febrile seizure history and family history, multiple seizure forms, cognitive language development delay, etc., it was consistent with the clinical characteristics of EMAS, and the gene detection results further verified our judgment.

CONCLUSION

SLC6A1 screening is recommended for children with multiple seizure types including atonic seizures, myoclonic seizures, GTCS and other seizures, developmental delay before the onset, with or without behavioral disorders, and seizure remission after drug treatment, so as to improve the prognosis of children. This study is individual and has limitations, the prevalence of each potential genetic etiology should be evaluated in a large multicenter cohort, and targeted testing of genes previously implicated in EMAS will help to further elucidate the yield of known genes and identify new candidates.

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Cite this article

Zhen L, Hui-Ping A, Chang-Ming H, Wei L, Yan-Chao MU (2022) SLC6A1 Gene Mutation Caused Epilepsy with Myoclonus-Atonic Seizures: A Case Report Clinical Characteristics and Genetic Analysis. J Autism Epilepsy 4(1): 1016.