CASE REPORT

Identification of a Novel *ASAH1* Gene Mutation in Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy

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ABSTRACT

Spinal muscular atrophy (SMA) with progressive myoclonic epilepsy (PME) affects the nervous system. Symptoms appear in early childhood and include muscle weakness, difficulty walking, seizures, and cognitive decline. Despite introducing various therapies to restore acid ceramidase function or reduce ceramide accumulation and gene therapy to correct genetic mutations, there are still unknown underlying molecular mechanisms related to this disorder. This article reports a novel variant c.118G>C in the ASAH1 gene. The patient presented with clinical manifestations such as progressive muscle weakness and myoclonic convulsions. Clinical features and electrophysiological investigations revealed a motor neuron disease and generalized epileptic discharge. A significant temporal interval was observed between the initial diagnosis of SMA and the subsequent manifestation of myoclonic seizures. The proband was genetically assessed through whole exome sequencing (WES) followed by variant confirmation and bioinformatics analysis. According to this article's findings and previous research, further diagnostic testing and management are needed to determine the severity and progression of the patient's condition.

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Introduction

Spinal muscular atrophy (SMA) with progressive myoclonic epilepsy (PME) is a rare genetic disorder impacting the nervous system (1). This complex condition manifests through a combination of SMA and PME (2). The symptoms of this disorder usually appear in early childhood and progressively worsen over time. Patients with this condition experience muscle weakness, difficulty walking, seizures, and cognitive decline. Despite the early onset of muscle symptoms, both in previously reported cases and in the present case, it has been observed that seizure symptoms manifest several years later. No cure is currently available for this disorder, and treatment is generally supportive (3).

The genetic basis of SMA-PME is associated with mutations in the *ASAH1* gene. This gene provides instructions for making an enzyme called acid ceramidase, which breaks down ceramide, a fat molecule. Mutations in the *ASAH1* gene lead to a deficiency of this enzyme, resulting in the accumulation of ceramide in the cells of the nervous system. This accumulation causes damage and dysfunction, leading to the symptoms associated with this disorder.

Despite being a rare disorder, SMA-PME is an area of active research. Researchers delving into the root causes of this disorder to create effective treatments. Some studies focus on creating treatments that can either rejuvenate acid ceramidase functionality or decrease ceramide buildup in cells. Additionally, significant investigations have been conducted into employing gene therapy to rectify the genetic mutations responsible for this disorder. This article reports a novel gene mutation in the *ASAH1* gene using exome sequencing followed by molecular techniques to identify the genetic cause of SMA-PME in an Iranian family.

Case presentation

A 10-year-old girl has been experiencing muscle weakness since the age of four. Her parents were consanguineous, with no similar phenotype in the family. She has two asymptomatic siblings, as shown in the pedigree. Five years ago, the patient underwent electromyography (EMG), which led to the diagnosis of SMA. Subsequently, multiplex ligament-dependent probe amplification (MLPA) was performed, but it yielded negative results. Thus, diagnostic tests were discontinued, and the patient received only occupational therapy without a definitive diagnosis. Six months after Whole Exome Sequencing (WES), the patient began to experience myoclonic jerks (approximately five years after the onset of muscle symptoms). Over the past eight months, she experienced limb myoclonus and head drop episodes, which were accurately diagnosed only upon referral to a neurologist. She was suspected of SMA-PME, and the following tests were performed comprehensively: a) Interictal electroencephalography (EEG) recordings during both wakefulness and sleep exhibited generalized bursts of high voltage slow waves in the brain (figure 1). b) Magnetic resonance imaging (MRI) revealed no other abnormalities. c) Antiseizure treatment was initiated with sodium valproate, which resulted in partial control of seizures. Nitrazepam was later added, and it almost wholly controlled the seizures. Currently, the patient is stable.

For genetic analysis, informed consent was obtained from the participants or their legal guardians. EDTA blood sample was collected from the patient and her parents. DNA was extracted from the blood sample using the Salting Out standard protocol to ensure high-quality DNA

extraction. WES was performed on the proband's DNA sample using the Illumine NovaSeq 6000 platform for 2*100 bp reads with an average coverage of 100X platform (Macrogen, Korea). Variants were called using the HaplotypeCaller algorithm in Genome Analysis Toolkit version 4.0 (GATK4) and annotated with ANNOVAR using various public and in-house databases. Functional analysis tools such as MutationTaster, PolyPhen2, and CADD were used to estimate the possible damaging effect of each variant on function and structure. Sanger sequencing on both parents and two other siblings was performed to confirm the variant. Specific primers were designed using online NCBI primer design, and PCR was performed to verify the identified variants. The sequencing data were analyzed according to the NCBI Gene reference sequence. Both parents were heterozygous, the healthy sibling was normal homozygous, and the proband was mutated homozygous (figure 1-B). However, an enzyme-level functional examination was not performed on the proband. Clinical and molecular findings are presented in Table 1.

Table 1. Clinical characteristics and molecular findings

Proband's age	10y
Sex	Female
Status	Alive
Parents' Consanguinity	+
Muscle weakness, proximal	+
Difficulty walking	+
Seizures	-
Serum creatine kinase	+
Gowers sign	+
Hypotonia	+
Cardiomyopathy	-
Nucleotide change	c.118G>C
Amino acid change	p.Gly40Arg
Exon	2
Zygosity	Mutated Homozygote
ACMG classification	VUS

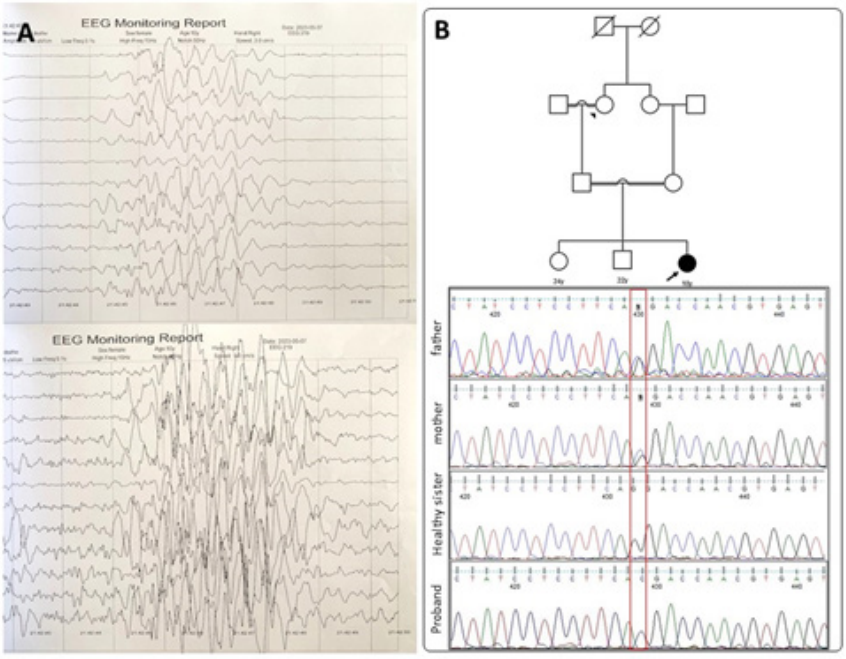

Fig 1. *Clinical and molecular findings in the case. A) EEG shows generalized bursts of slow-vivo voltage waves in the brain. B) Pedigree and chromatogram for ASAH1 c.118G>C variant. The black shape represents the affected individual; the arrow indicates the proband*

Discussion

This article reports a novel variant c.118G>C in the *ASAH1* gene, which leads to the replacement of arginine at the codon 40 (p.Gly40Arg) located in the disulfide bond domain. This bond latches the N-terminal end of the α-subunit linker to the β-subunit (C31/C340) covalently (4). Some patients with the SMA-PME phenotype have been found to carry mutations in the *ASAH1* gene. To date, fifty-eight cases of SMA-PME have been reported, out of which thirty-seven cases have been confirmed through genetic testing (5). In the present case, a temporal interval of four to five years was observed between the initial diagnosis of muscle disease and the manifestation of seizure symptoms. However, the initial negative result of the SMA test led to the discontinuation of further diagnostic measures, resulting in a definitive diagnosis being made five to six years after the onset of muscular symptoms.

In 1979, Jankovic and Rivera were the first to report the connection between distal muscular atrophy and myoclonic seizures in a large four-generation family. The affected individuals displayed adult-onset, generalized, stimulus-sensitive myoclonus, and slowly progressive distal muscle weakness and wasting. Some patients lived into their sixties, while others developed dementia. Clonazepam was effective in controlling the seizures. A postmortem examination of one patient revealed neuronal degeneration in the anterior horn cells, Clarke nucleus, and lower cranial nerve nuclei. The transmission pattern of the disorder in this family was consistent with autosomal dominant inheritance (6). However, the first report of SMA associated with PME linked to *ASAH1* was published in 2012 (7).

All reported cases of SMA-PME had muscle weakness and wasting, myoclonic jerks, and seizures, regardless of differences in age of onset, severity, and progression of the disease. Other reported less consistent features include tremors, cognitive impairment, sensorineural hearing loss, and cerebral and cerebellar hemispheres atrophy. The onset of the disease is characterized by muscle wasting and weakness in early childhood, followed by epilepsy after the onset of motor symptoms. *ASAH1* mutations are also associated with a more severe form of the disease with ceramidase activity less than 10%, known as Farber lipogranulomatosis (FL), but joint and skin findings of FL are not reported in SMA-PME (1, 7-9).

Acid ceramidase (ACDase) deficiency is a spectrum of disorders, including Farber disease (FD) and SMA-PME—mutations in the ASAH1 gene cause the conditions mentioned above. The clinical presentation of FD and SMA-PME is variable, and treatment is mainly planned based on symptom management. Research is being conducted to evaluate the use of recombinant human ACDase for treating FD, and gene therapy strategies are being pursued. The review provides insights into the pathology and possible biomarkers to diagnose ACDase deficiency effectively (1).

In Conclusion

Based on the clinical findings, in patients with an EMG diagnosis of SMA disease but with negative MLPA results, it is essential to gather a detailed history of myoclonic jerks occurring during sleep and wakefulness. Furthermore, if there is strong evidence of seizure activity, obtaining an EEG and performing WES is recommended to investigate the possibility of genetic mutations. Additionally, for patients diagnosed with SMA but with negative MLPA results, it is advisable to consider skin,

joint, and larynx examinations, particularly for FD. Further diagnostic testing and management will be necessary to determine the severity and progression of the patient's condition. Close monitoring and supportive care will be crucial in managing the patient's symptoms and improving their quality of life. Genetic counseling may also be recommended for the patient's family, given the increased risk of SMA in consanguineous relationships.

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Author's Contribution

Meisam Babaei: Clinical examination, Data collection & Study design. Najmeh Ahangari: Data analysis & manuscript drafting. Fatemeh Arab: Genetic testing and Data analysis.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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