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CASE REPORT

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A Novel Disease-Causing ASPA Gene Mutation (c.432+1 G>C) in an Iranian Patient with Canavan Disease: A Case Report*

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Abstract

Canavan disease is an autosomal recessive genetic disease and rare fatal childhood neurological disorder caused by mutations in the *ASPA* gene, which resulted in a catalytic deficiency of the *ASPA* enzyme that catalyzes the hydrolysis of N-acetylaspartic acid into aspartate and acetate. Herein, we report an Iranian patient diagnosed with Canavan disease with a novel splice-site mutation in the *ASPA* gene (NM_000049.4; c.432+1 G>C). This report is based on a homozygous c.432+1 G>C mutation in the *ASPA* gene identified from an Iranian patient. As a result, a novel homozygous pathogenic mutation on *ASPA* is the cause of disease in the patient.(**International Journal of Biomedicine. 2021;11(4):594-597.**)

Key Words: Canavan disease • novel mutation • ASPA gene • aspartoacylase • N-acetylaspartic acid

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Abbreviations

NAA, N-acetylaspartic acid; **MRS**, magnetic resonance spectroscopy; **MRI,** magnetic resonance imaging; **gDNA**, genomic DNA; **WES,** whole-exome sequencing; **ACMG**, American College of Medical Genetics and Genomics.

Introduction

Canavan disease is an autosomal-recessive leukodystrophy and fatal neurological disease which is characterized by developmental delay, neurologic deterioration with severe intellectual disability, and early death.(1) The underlying cause of this disease is the deficiency in the enzyme aspartoacylase, which leads to high levels of N-acetylaspartic acid (NAA) in the urine, brain, and body fluids. The *ASPA* gene mutations are responsible for this deficiency (RefSeq NM_000049.4).(2) ASPA is a catabolic enzyme that is primarily in oligodendrocytes in the central nervous system.(3) ASPA catalyzes the hydrolysis of NAA to generate aspartate and acetate, it is a homodimer and essential in the synthesis of myelin. Patients who are deficient in the ASPA enzyme activity have an abnormal elevation of NAA in the brain. This can be identified by applying magnetic resonance spectroscopy (MRS) even before increasing its concentration in the urine, which is suitable for the early diagnosis of Canavan disease.(4) Clinical symptoms are not manifested at the time of birth; however, the clinical triad of hypotonia, macrocephaly, and head lag often in association with macrocephaly and

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seizures are initial diagnostic manifestations for Canavan disease in early childhood.[5] Neuroimaging presents brain white matter signal abnormalities, and, at later time-points, ventricular enlargement.[6,7] Patients with Canavan disease in the first months of life have dysmyelination, intramyelinic edema, and characteristic spongiform degeneration of the white matter of the brain with impairment of psychomotor development, which is specified by cognitive delay, ataxia, and irritability. In atypical cases, disease onset is postponed until some years after birth when some aspartoacylase enzymatic activity remains.[8,9] Other symptoms involve difficulties in sucking and visual tracking, progressive macrocephaly, and preserved social interactions. Disease progression is marked by atrophy of the optic nerve, spastic tetraparesis, intellectual disability, seizures, and early death. Magnetic resonance imaging (MRI) is routinely used for the diagnosis of the characteristic spongy degeneration of the white matter, which typically shows signal abnormalities of the white matter and the basal ganglia. Up to now, the Human Gene Mutation Database has presented 83 mutations in the *ASPA* gene (www.hgmd.cf.ac.uk). Novel mutations in some genes can be inherited in an autosomal dominant or autosomal recessive inheritance.[10,11] Herein, we present a novel mutation in the ASPA gene in one patient in an Iranian family with severe Canavan disease.

Case presentation

Our patient was the second born male child (Fig. 1A; IV:2) of consanguineous parents with no family history of any genetic condition or mental retardation. The parents have two children. The father and mother are first cousins and showed no signs or symptoms of Canavan disease. The 6-year-old girl is healthy and the second child is a boy with 2 years old and has symptoms of Canavan disease. The mother started to notice a delay in acquiring developmental milestones by the age of 4 months as he has the symptoms of hypotonia. At 19 months of age, he had severe developmental delay, a lack of neck support, frontal bossing, and macrocephaly. MRI of the brain revealed a diffuse lesion of the white matter affecting the U-fibers. The diagnosis of Canavan disease was confirmed by the findings of a very high concentration of NAA in urine. The parents of the patient provided written informed consent in accordance with the Helsinki Declaration.

A salting-out method for genomic DNA (gDNA) extraction was done. gDNA sample from peripheral blood lymphocyte of the patient was examined by whole-exome sequencing (WES) technique (Macrogen, Seoul, South Korea) to identify the mutations associated with this disease. A novel homozygous ASPA c.432+1 G>C mutation in exon2/intron2 boundary region (NM_000049.4) was found. Finally, to confirm the presence of the *ASPA* variant, a direct Sanger sequencing method for the patient and his parents were done. So, the specific sets of primers were designed to amplify the mutated sites in the genome by the PCR method. After amplification of *ASPA* sequences, we sequenced the PCR products directly on the automated genetic analyzer (ABI 3130XL; USA) and the results are represented in Figure 1B. This finding has not been reported in the other Canavan patients.

Furthermore, the splice-site mutation (c.432+1 G>C) was classified as pathogenic according to the American College of Medical Genetics and Genomics (ACMG) guidelines. MutationTaster (bioinformatics program) predicted that this mutation is disease-causing.

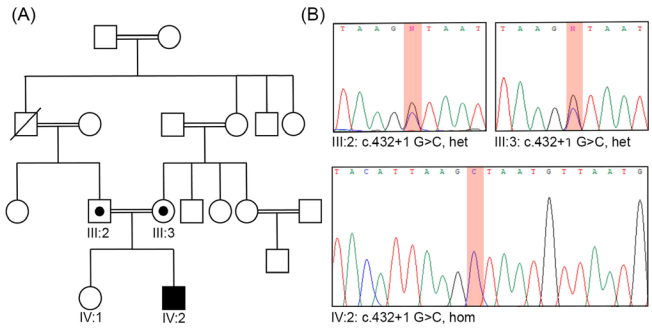

Fig 1. *(A) Family pedigree of the patient. The black square represents the patient. Parents have a family history of consanguinity. The unfilled square represents a male, and the circle represents a female. The diagonal line on the square represents a deceased male. The circle in the center of symbols represents heterozygosity. (B) Sequence analysis of the patient and parents revealed that in terms of inheritance, the patient's parents were heterozygous (III:2 and III:3) and the patient (IV:2) was homozygous. The chromatogram shows the splicing mutation, G to C change in the splice donor of intron 2 (c.432+1 G>C).*

Discussion

Known as Canavan disease, it is an autosomal recessive form of human spongiform leukodystrophy caused by an inborn error of the *ASPA* activity.[12] The substrate of aspartoacylase enzyme is NAA, which is exclusively synthesized in the brain. NAA is hydrolyzed by aspartoacylase to acetate which is necessary for myelin synthesis and aspartate.[13,14] Aspartoacylase deficiency leads to accumulation of NAA in the brain, causing pathological spongy degeneration of the white matter.[14,15] Clinically, two types of Canavan disease have been reported. The most common type of Canavan disease is the neonatal or infantile form that is more severe in clinical presentation in comparison with the juvenile type of the disease.[16,17] Clinical symptoms of neonatal or infantile Canavan disease commonly begin between the age of 2 to 6 months and appear with lack of neck holding in pull to sit maneuver, axial hypotonia, lethargy, spasticity as well as macrocephaly, poor feeding, developmental regression, and progressive hyperreflexia. Cortical blindness and optic atrophy are followed by seizures in later stages.[17] The clinical course of our patient was consistent with the infantile type of Canavan disease.

Extraction of human cDNA and the *ASPA* gene helps to explain the molecular basis of Canavan disease. *ASPA* is the only gene for Canavan disease that is located on chromosome 17p13.2.[16] The examination of mutations in patients with Canavan disease has revealed missense, nonsense, splice-Site, and frameshift mutations, deletions, or insertions.[18] Canavan disease is more prevalent in people of Ashkenazi Jewish than another ethnicity.[19]

Several mutations have been reported to have phenotypes associated with their genotypes, such as 698insC, X314W,

P181L, 244delAT, 923delT, C152W, V14G, D249V, and E214X with a severe phenotype. A stop codon or frameshift occurs in many of these mutations that is related to the onset during the first few months after birth (2 or 3 months of age). In Jewish populations, E285A and Y231X mutations are correlated with a severe phenotype as well.[20] The mutation that was found in our patient also cause a severe phenotype.

Deficiency of the *ASPA* activity caused by the nonsense tyr231ter, the missense ala305glu mutation, or the glu285ala mutation establishes that the three coding-sequence mutations are the cause of Canavan disease.[21] The 433-2 A to G transition in intron 2 (in the splice acceptor site) would result in skipping of exon 3. Additionally, skipping of 94-base exon 3, in the final transcript will change the reading frame. A frameshift accompanied by an exon-skipping would result in the aspartoacylase deficiency.[22]

In 2012, Durmaz et al. reported a novel heterozygous mutation Y88X (T to A nucleotide change at codon 88 in exon 2) within the aspartoacylase gene in a consanguineous family with an affected child diagnosed as Canavan disease. This mutation converts the codon for tyrosine (TAT) into a premature termination codon (TAA).[20] Also, in 2015, Ashrafi et al. indicated a novel homozygous missense mutation (c.202G>A) in the *ASPA* gene in exon 1 which was found in an Iranian patient.[23] In our case, we presented a novel homozygous pathogenic mutation in *ASPA* gene (c.432+1 G>C) related to Canavan disease. This mutation was at the 5' splice-site beginning intron 2, which can cause mis-splicing and alter the reading frame, and consequently, it will probably result in a serious alteration in ASPA protein conformation and leads to the Canavan phenotype. This type of mutation has not been reported in other populations.

Conclusion

In the present study, we report a 2-year-old Iranian boy with severe Canavan disease who harbors a novel pathogenic homozygous mutation (c.432+1 G>C) in the *ASPA* gene. Homozygous mutation as in the intron 2 of *ASPA* gene in the present case is a novel splice-site mutation that was not reported elsewhere. The mutation that leads to the Canavan disease has been defined in the family; it would make prenatal diagnosis possible and suggest parents with such disorder plan for the next pregnancy.

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Competing Interests

The authors declare that they have no competing interests.

Disclaimers

The views expressed in this article are the author's own and do not reflect the official position of the institutions.

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