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Case Report

A Novel Homozygous Variant in the *Aspartoacylase* Gene Causes Canavan Disease-Case Report

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ABSTRACT

Glu178 is the active site residue essential for substrate affinity and catalytic activity of the aspartoacylase enzyme. Sanger sequencing in an infant with Canavan disease revealed a homozygous ASPA: c.532G>A: p. (Glu178Lys) variant. Glu178Lys is the first ever variant reported at the critical active site of aspartoacylase protein and this variant might significantly disrupt substrate interaction.

Keywords: Aspartoacylase, brain, Canavan disease, mutation, myelin, N-acetylaspartate

Introduction

Canavan disease (CD) is a rare and serious autosomal recessive neurodegenerative disorder associated with spongy degeneration of the white matter of the brain. It is caused by a mutation in the aspartoacylase (*ASPA*) gene leading to the loss of or reduced ASPA enzyme activity (1,2). This disease has been reported worldwide, but is more often seen in Ashkenazi Jewish populations. The estimated incidence of severe CD in the non-Jewish population is about 1:100,000 births (3). The estimated prevalence of CD in the Arab world ranges from 1:6,000 to 1:14,000 (4). The prevalence of CD in India is not known. The ASPA protein comprises 313 amino acids with an approximate molecular weight of 36 kilo Daltons. It forms a dimer with zinc at the catalytic site and facilitates the hydrolysis of N-acetyl L-aspartate (NAA) into aspartic acid and acetate. The *ASPA* gene is located on the short arm of chromosome 17 and comprises 29 kilobases with six exons and five introns. The lack of ASPA activity leads to demyelination resulting from the accumulation of NAA in the brain. The phenotype ranges from severe typical to less severe atypical CD. Typical CD, the most common type, manifests with neurodevelopmental impairment by three to five months of age, followed by progressive neurodevelopmental regression. Atypical CD usually manifests with neurodevelopmental delay in the first years of life, followed by developmental regression in childhood or adolescence and has a more variable clinical course than typical CD. Genotype-phenotype correlations have been proposed depending on the effect of variants on the residual ASPA. Pathogenic variants p.Tyr231Ter

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and p.Glu285Ala, which are most commonly seen in the Ashkenazi Jewish populations, are associated with typical CD. The pathogenic variant p.Ala305Glu, commonly seen in European individuals without Ashkenazi Jewish ancestry, manifests with both typical and atypical CD. Pathogenic variants p.Arg71His, p.Asp204His, p.Pro257Arg and p.Tyr288Cys are associated with atypical CD. The usual findings on magnetic resonance imaging (MRI) studies of the brain are diffuse, symmetric white matter changes, especially in the subcortical and cortical areas. The MR spectrometry (MRS) to detect NAA has been reported as being the best method for the diagnosis of CD in infants (1). The molecular diagnosis of CD can be achieved using Sanger sequencing for the sequencing of all exons of the *ASPA* gene. Exome sequencing can also be used to identify variants in other genes which might be responsible for causing phenotypes similar to CD. Classical clinical features and elevated NAA in urine and/or with bi-allelic pathogenic variants in ASPA identified by molecular genetic testing confirm the diagnosis of CD in a proband. Currently, the treatment is mainly supportive as there is no specific treatment, and the prognosis is guarded as the majority of cases with CD die in the first decade of life (1). We report a case of novel homozygous variant at the Glu178Lys residue, a critical active site of the *ASPA* gene leading to CD.

Case Report

A 9-month-old girl, second-born to a second-degree consanguineous marriage, was admitted with a history of delay in attaining age-appropriate milestones in all domains. The mother had observed that the infant had no head control by 6 months of age. Also, the infant did not turn the head to sound nor fix and follow light. The infant recognized the mother by 7 months of age. The infant had no history of convulsions or abnormal movements. The family history was insignificant except for consanguineous marriage among the parents of the infant. The elder sibling, a seven-year-old male, did not have any symptoms of the disease. The infant had an uneventful perinatal history. On examination, her weight, length, and head circumference were 5.7 kg (<-3SD), 65 cm (<-2SD) and 47 cm (>+2SD) respectively. She had macrocephaly (Figure 1) with an open anterior fontanelle (2x2 cm). Her developmental assessment revealed global developmental delay. She had no head control, social smile or visual fixation. She could not hold objects in her hand. The infant inconsistently turned her head to sound. The infant had generalized hypotonia (central more than peripheral) and intermittent hypertonia in all four limbs. A detailed ophthalmological examination revealed bilateral optic atrophy. Hearing assessment with distortion product oto-acoustic emissions revealed bilateral presence of emissions. Brainstem evoked response audiometry could not be carried out as the test was not available at our centre. The MRI of the brain revealed flair and T2 hyper intensities in the internal capsule, subcortical fibers, and bilateral cerebral and cerebellar hemispheres. The MRS demonstrated markedly elevated NAA. Molecular analysis was performed using DNA extracted from the blood samples of the proband and her mother, obtained after informed consent, followed by amplification of all exons of the *ASPA* gene. Sanger sequencing of the amplified exons revealed a heterozygous ASPA: c.532G>A:p..-(Glu178Lys) variant in the exon-4 region (Figure 2a) in the mother, consistent with carrier status, and a homozygous variant in the proband aligning with an autosomal recessive inheritance. Sanger sequencing was not performed on the father as he did not consent to undergo the test. Molecular docking using Auto Dock Vina revealed distinct interaction profiles for the wild type and Glu 178Lys variant of ASPA with substrate AS9 (5,6). 2D and 3D interaction maps show that the wild type engages robustly through hydrogen bonds and ionic interactions centered around Glu178, supporting a strong binding affinity of -6.3 kcal/mol. In contrast, the Glu178Lys variant, with a reduced affinity of -5.7 kcal/mol, displays an altered binding landscape with fewer hydrogen bonds and increased hydrophobic contacts, indicating the variant's destabilizing effect on substrate binding (Figure 2b). A final diagnosis of CD was made, and the parents were provided

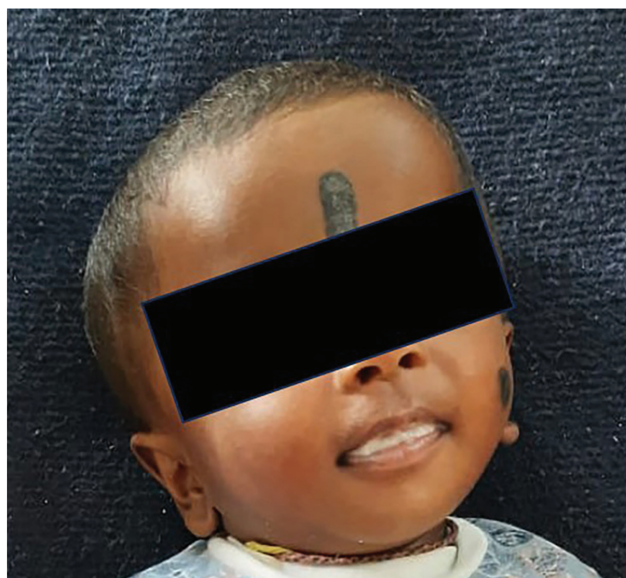

Figure 1. Macrocephaly in the infant

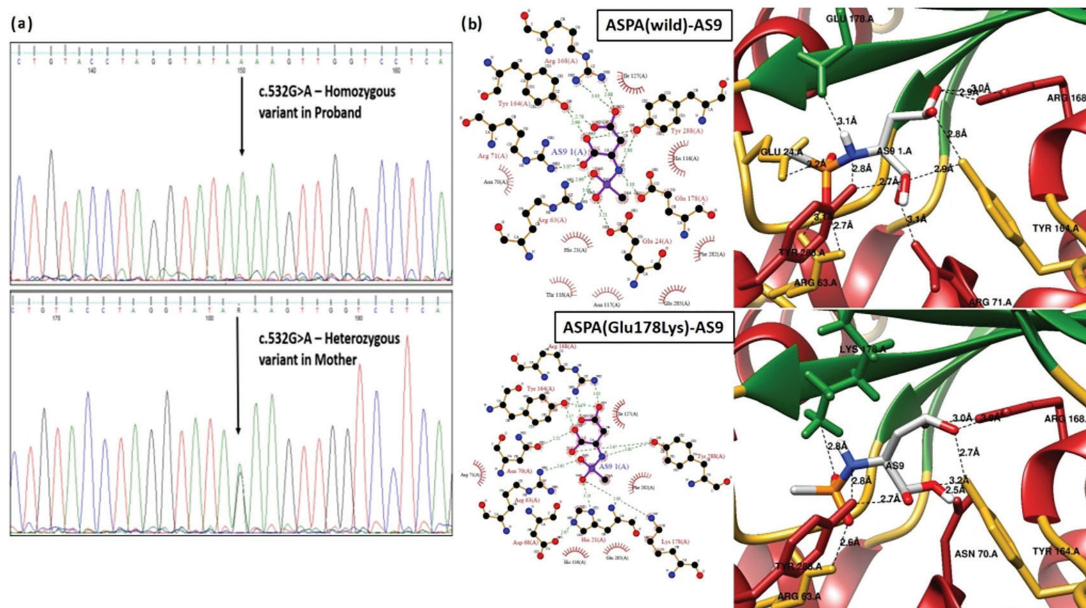

Figure 2. Genetic and molecular interactions in ASPA. (a) Sanger sequencing chromatograms displaying the c.532G>A variant in ASPA gene (b) Comparative molecular interaction maps of *ASPA*-wild type (top panel) and ASPA-Glu178Lys variant (bottom panel) with substrate AS9, including 2D interaction diagrams (left) and 3D representations (right)
ASPA: Aspartoacylase

genetic counseling. The infant was registered at the district early intervention center in our institute and provided with supportive care. The infant was lost to follow-up.

Discussion

Infants with severe forms of CD usually manifest with hypotonia, macrocephaly, and developmental delay by three to five months (1). The usual MRI findings include diffuse, symmetrical white matter changes predominantly in the subcortical and cortical areas. It is reported that detection of NAA by MRS is the prime method for the diagnosis of CD in infants (1). This infant had the classical clinical and radiological features suggestive of CD.

The ASPA protein, pivotal in CD pathogenesis, displays two-domain architecture. The N-terminal domain is structurally composed of a six-stranded β-bundle surrounded by eight α-helices, while the C-terminal domain predominantly consists of β-sheet and coil structures wrapping around the N-terminal domain. The ASPA protein family is characterized by conservation of four identical residues Asn70, Asp114, His116, and Glu178 across the 33 seed members, in addition to other conserved residues such as His21, Gly22, Glu24, Asn54, Arg63, Arg71, and Phe73 located near the active site (7). Notably, the active site, critical for its enzymatic function, is constituted by residues from the N-terminal domain with a particular emphasis on the conserved Glu178. This residue acts as a general base to activate a nucleophilic water molecule coordinated to the zinc ion, thereby facilitating the release of the L-aspartate product from the enzyme (8).

The gene coding for ASPA has been mapped to chromosome 17p13-ter (2). More than 70 different human *ASPA* gene mutations have been reported (9). The majority are missense mutations located remotely from the catalytic site. About 60% of the known missense mutations are located within the N-domain. A study (10) observed c.162 C>A (p.Asn54Lys), c.859 G>A (p.Ala287Thr), c.728 T>G (p.Ile243Ser) and c.902 T>C (p.Leu301Pro) mutations in CD patients from the Indian subcontinent. In this case, the variant observed was ASPA: c.532G>A:p.(Glu178Lys). Gene therapy is an emerging treatment approach aimed towards curing CD (11). This case emphasizes the importance of genetic testing. Precise knowledge of the mutation type is critical in aiding tailored gene therapy approaches, potentially improving their efficacy and safety. In this case, the disparity in the binding characteristics of the variant ASPA suggests a significant alteration in substrate affinity which might contribute to the pathogenesis of CD. Glu178Lys, the first ever variant reported at the critical active site of ASPA, could potentially lead to a substantial reduction in substrate binding efficiency, reducing overall enzymatic function. We report a novel variant at the Glu178Lys residue of ASPA, which could expand the opportunity for tailored treatments and therapies in CD.

Ethics

Informed Consent: Molecular analysis was performed using DNA extracted from the blood samples of the proband and her mother, obtained after informed consent, followed by amplification of all exons of the *ASPA* gene. Written informed consent has beenobtained from the parents of the child for publishing this case report.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.V.K., S.K.L., D.K., K.S., K.R.Y., M.K., T.P.L., A.D., Concept: S.K.L., Literature Search: A.V.K., S.K.L., D.K., K.S., K.R.Y., M.K., T.P.L., A.D., Writing: A.V.K., S.K.L., D.K., K.S., K.R.Y., M.K., T.P.L., A.D.

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References

1. Nagy A, Bley AE, Eichler F. Canavan Disease. 1999 [updated 2023 Dec 21]. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024.
2. Kaul R, Balamurugan K, Gao GP, Matalon R. Canavan disease: genomic organization and localization of human ASPA to 17p13-ter and conservation of the ASPA gene during evolution. Genomics. 1994; 21:364-70.
3. Orphanet. Canavan disease. Orphanet Journal of Rare Disease [Internet]. 2012. https://www.orpha.net/en/disease/detail/314911
4. Zayed H. Canavan disease: an Arab scenario. Gene. 2015; 560:9-14.
5. Eberhardt J, Santos-Martins D, Tillack AF, Forli S. AutoDock Vina 1.2.0: new docking methods, expanded force field, and python bindings. J Chem Inf Model. 2021; 61:3891-8.
6. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010; 31:455-61.
7. Le Coq J, Pavlovsky A, Malik R, Sanishvili R, Xu C, Viola RE. Examination of the mechanism of human brain aspartoacylase through the binding of an intermediate analogue. Biochemistry. 2008; 47:3484-92.
8. Bitto E, Bingman CA, Wesenberg GE, McCoy JG, Phillips GN Jr. Structure of aspartoacylase, the brain enzyme impaired in Canavan disease. Proc Natl Acad Sci U S A. 2007; 104:456-61.
9. Stenson PD, Mort M, Ball EV, et al. The human gene mutation database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis, and next-generation sequencing studies. Hum Genet. 2017; 136:665-77.
10. Bijarnia S, Kohli S, Puri RD, et al. Molecular characterisation and prenatal diagnosis of aspartoacylase deficiency (Canavan disease): report of two novel and two known mutations from the Indian subcontinent. Indian J Pediatr. 2013; 80:26-31.
11. Grønbæk-Thygesen M, Hartmann-Petersen R. Cellular and molecular mechanisms of aspartoacylase and its role in Canavan disease. Cell Biosci. 2024; 6:45.