CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

A 37-Year-Old Man With Intellectual Disability Discovered to Have Aspartylglucosaminuria

Implications for the Diagnosis of Genetic Causes

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Neurol Genet 2024;10:e200161. doi:10.1212/NXG.0000000000200161

Abstract

Objectives

The causes of intellectual disability (ID) are varied, with as many as 1,400 causative genes. We attempted to identify the causative gene in a patient with long-standing undiagnosed ID.

Methods

Although this was an isolated case with no family history, we searched for the causative gene using trio-based whole-exome sequencing (trio-WES), because severe ID is often caused by genetic variations, and inherited metabolic disorders (IMDs) are assumed to be the cause when regression and epilepsy occur.

Results

We identified homozygous donor splice-site variants in the *AGA* gene (aspartylglucosaminidase; NM_000027.4) Chr4(GRCh38):g. 177436275C>A, c.698+1G>T. This gene is implicated in aspartylglucosaminuria (AGU; OMIM #208400) and originated from both of the patient's parents. We confirmed the pathogenicity of the variant by detecting the splicing defect in cDNA from the patient's blood and accumulation of aberrant metabolites in the patient's urine.

Discussion

We discuss how to more readily achieve an accurate diagnosis for patients with undiagnosed intellectual disabilities. Medical practitioners' awareness of the characteristics of the disease leading to clinical suspicion in patients with matching presentations, and the performance of newborn screening when possible, is important for the diagnosis of ID. In addition, the characteristic symptoms and course of the disease give rise to suspicion of IMDs. Given our results, we consider trio-WES to be a powerful method for identifying the causative genes in cases of ID with genetic causes.

Introduction

Intellectual disability (ID) is defined as a developmental disability that includes deficits in both intellectual and adaptive functioning in conceptual, social, and practical domains. It is a relatively common condition that occurs in 1%–3% of the population, and severe ID is highly associated with genetic causes. While genetic abnormalities in many IDs are difficult to treat at this time, IMDs constitute a subgroup of rare genetic conditions for which an increasing number of treatments have become

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Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

available. We succeeded to identify the causative gene in a patient with long-standing undiagnosed ID caused by an inherited metabolic disorder, and we present the lessons learned from this case.

Case Presentation

A 37-year-old man with psychomotor retardation was admitted to our hospital because of status epilepticus and aspiration pneumonia. He was born to reportedly nonconsanguineous parents, and there was no family history of ID. He was born at 39 weeks' gestation with a normal birth weight of 3,030 g. He achieved independent walking at the age of 1 year and 4 months and significant speech at 1 year and 6 months. Such a developmental delay is often observed as the first neurologic sign of aspartylglucosaminuria (AGU).[1] He had recurrent diarrhea, which is a characteristic feature of AGU. In elementary school, he attended a special needs class. Around 12 years of age, his verbal and intellectual abilities started to regress. He underwent 2 hospitalizations due to aspiration pneumonia at the ages of 27 and 34 years, and his motor regression progressed markedly over that time span, eventually leaving him wheelchair bound. Similarly, his speech declined, eventually to the point of mutism. At the age of 35 years, he developed medication-resistant epileptic seizures.

On admission, his appearance was cachexic due to long-term malnutrition, and he had coarse facial features with a high forehead, thick eyebrows, hypertelorism, and a wide nose bridge (Figure 1A). He was missing both of his middle fingers due to osteomyelitis caused by previous self-biting. He had scoliosis (eFigure 1A). Neurologic examination showed cognitive decline with loss of speech and disuse atrophy in all extremities, although he was able to direct his attention to stimuli.

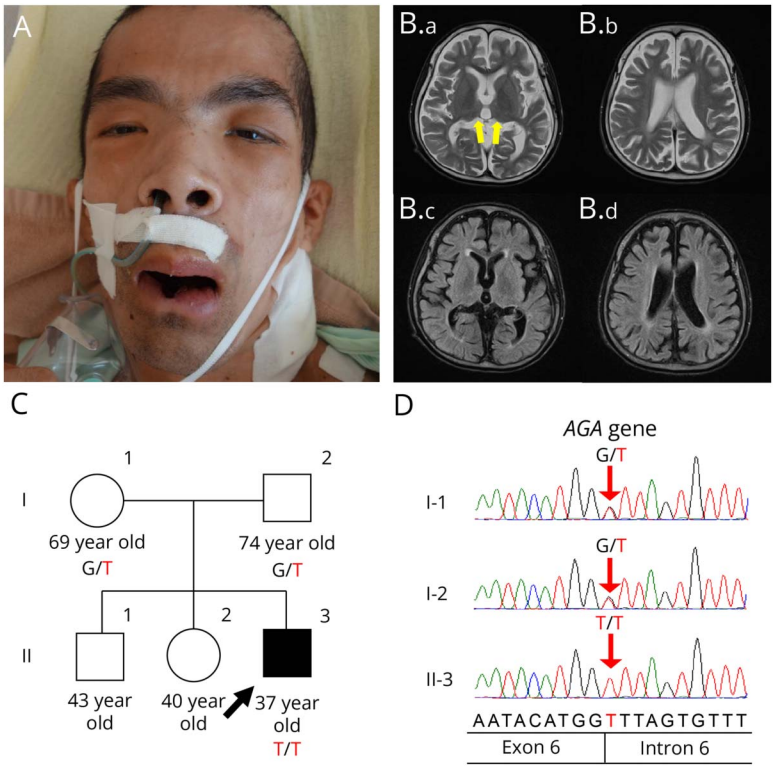
An EEG showed slow waves with frontal lobe predominance. Abdominal CT showed splenomegaly (eFigure 1B). His skull was thick (eFigure 1C). Brain MRI showed mild atrophy of the bilateral frontal lobes, mild T2 hypointensities in the pulvinar nuclei of the thalami, and mild T2/FLAIR hyperintensity in the periventricular white matter (Figure 1B.a–d).

During the patient's hospitalization, 2 medical issues arose. First, the patient developed recurrent infections, with repeated bouts of pneumonia, urinary tract infection, and bacteremia throughout his hospital course. Second, the patient exhibited malabsorption, with repeated vomiting hindering his oral intake. Despite the tube feeding, persistent vomiting caused malnutrition and led to hypoglycemic attacks. He developed septic shock and died 6 months after admission.

Genetic Analysis

Trio-based whole-exome sequencing (trio-WES) was performed with the approval of the Ethics Committee of Kyoto University

Figure 1 Clinical Features, Pedigree, and Genetic Analysis

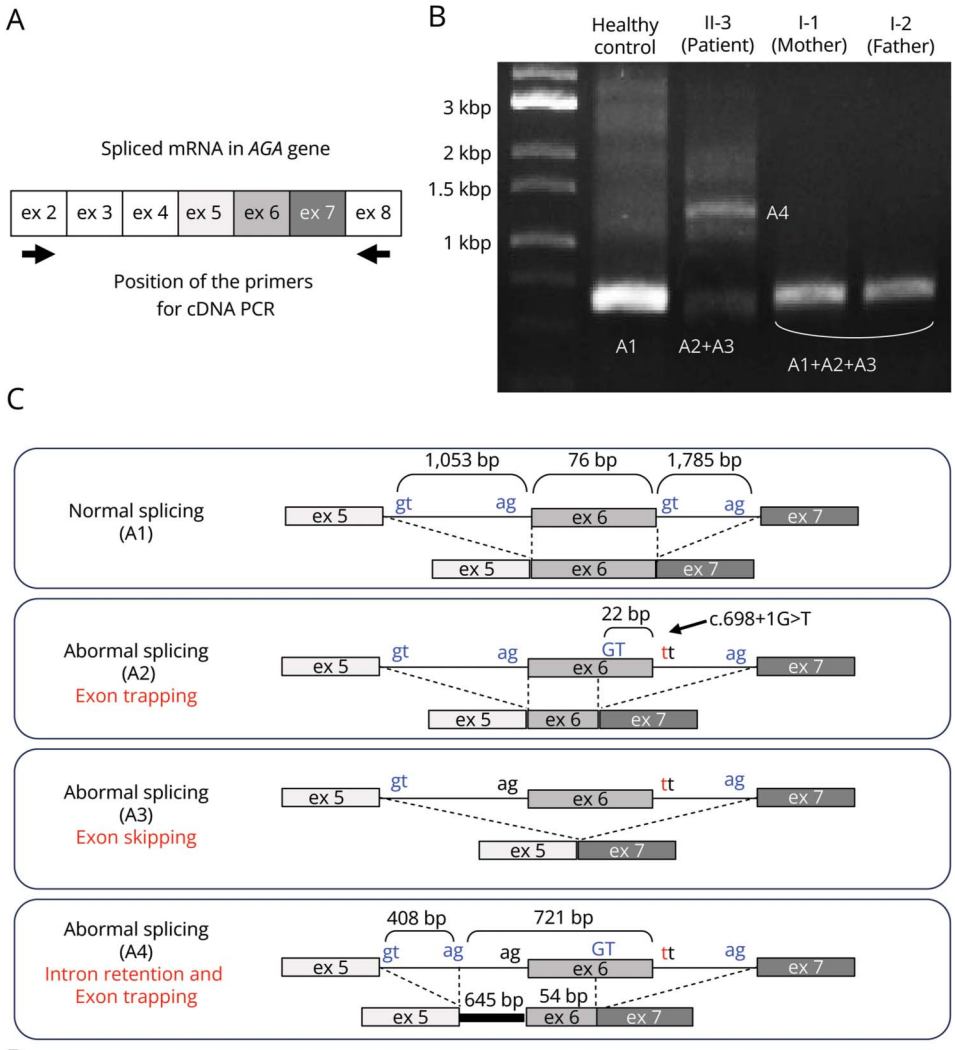


(A) The patient's facial appearance showing coarse facial features with a high forehead, thick eyebrows, hypertelorism, and a broad nasal bridge. (B.a to d) Brain MRI showing mild atrophy of the bilateral frontal lobes, mild T2 hypointensities in the pulvinar nuclei of the thalami (a, arrows), and mild T2/FLAIR hyperintensity in the periventricular white matter (b, d). (C) Family pedigree. The filled symbol indicates the patient, and the arrow indicates the proband. Blood samples were obtained from the mother (I-1), the father (I-2), and the patient (II-3), and analyzed by trio-WES. (D) Sanger sequencing of *AGA*.

Graduate School and Faculty of Medicine (Approval number: G1233) (eMethods). WES identified homozygous donor splice-site variants in *AGA* (NM_000027.4) Chr4(GRCh38): g.177436275C>A, c.698+1G>T, which originated from both parents (Figure 1C). Pathogenic variants in the *AGA* gene cause AGU.[1] We confirmed the variants by Sanger sequencing (Figure 1D). The allele frequency of the variant was examined by gnomAD (The Genome Aggregation Database),[2] and the variant was very infrequent (0.00001099; eTable 1) with no homozygotes. The predicted functional effect of the variant was evaluated using an in silico tool, Combined Annotation-Dependent Depletion (CADD).[3] Both the CADD raw score (5.9) and the PHRED-scaled CADD score (33) were high enough for confident pathogenicity prediction (eTable 1).

We analyzed the effect of the variant on splicing (eMethods). The results showed that the length of the patient's main cDNA PCR product from *AGA* was abnormally extended, including the last 645 bp of intron 5 (intron retention) and lacking the last 22 bp of exon 6 (exon trapping) (A4;

Figure 2 cDNA Analysis of *AGA*



D

Uploaded variant	Δ type	Δ score	Position	REF score	ALT score	Confirmed transcripts
chr4: 177436275 C>A or NM_000027.4 (AGA):c.698+1 G>T	Acceptor loss	0.19	76 bp	0.52	0.33	A3, A4
	Donor loss	0.37	1 bp	0.37	0.00	A2, A3, A4
	Acceptor gain	0.01	721 bp	0.23	0.24	A4
	Donor gain	0.03	23 bp	0.27	0.30	A2, A4

(A) Position of the primers for cDNA PCR. (B) Electrophoresis image of the cDNA PCR product in the patient, the parents, and healthy control samples. (C) Schematic depiction of structural variations in each splicing product. A1 to A4 correspond to those in Figure 2B. (D) *In silico* prediction of aberrant splicing by the variant with SpliceAI. The higher the Δ-score, which is calculated by the REF score minus the ALT score, the more likely the splice change is to be validated. The column marked with Position indicates base-pair distance from the variant position (c.698 + 1) to the newly predicted splice site toward the 5′direction. The column marked with Confirmed transcripts indicates cDNA PCR products that were validated for the newly predicted splice site.

Figure 2B-C). By contrast, the maternal and paternal cDNA PCR products contained a mixture of both normal and abnormal splicing products, but with a greater amount of normal splicing product and without the A4 cDNA PCR product (A1, A2, A3; Figure 2, B and C). A detailed cDNA sequence analysis is attached (eFigure 2).

After the cDNA analysis, we tried evaluating the performance of in silico prediction with SpliceAI (eMethods). Of interest, SpliceAI predicted the results with complete accuracy (Figure 2D).

Biochemical Analysis

Patients with AGU excrete aberrant urinary oligosaccharides. Therefore, urinary oligosaccharide excretion profiles were evaluated, as has recently been published.[4] Briefly, oligosaccharides in underivatized urine samples were chromatographically separated by ultra-high-performance liquid chromatography (UHPLC), followed by high-resolution accurate mass (HRAM) mass spectrometry analysis in negative ionization mode. Data were processed by a custom bioinformatics analysis pipeline from which age-dependent Z-scores of AGU-specific biomarkers were calculated. Investigation of patient urinary oligosaccharide excretions demonstrated highly elevated levels of the AGU-specific biomarkers Asn-GlcNAc, Asn-GlcNAc-Gal, and Asn-GlcNAc-Gal-NANA, with Z-scores of 632, 994, and 379, respectively (squares in Figure 3, upper table). The Z-scores of the patient sample were extremely higher than the Z-scores of the control sample (squares in Figure 3, lower table). The aberrant glycoasparagines were not detectable in the control urine sample and were also the highest compared with other representative patients with AGU (circles in Figure 3, upper and lower tables).

In addition, patient and control urine samples were subjected to routine amino acid analysis using ion exchange chromatography according to the manufacturer's recommended instructions (Biochrom analyzer, Biochrom, Cambridge, UK), which revealed increased excretion of undegraded aspartylglucosamine (208 mmol/mol creatinine), which was not detectable in the control urine sample.

Discussion

AGU, which is prevalent in the Finnish population, is a severe and progressive autosomal recessive lysosomal storage disorder. The deficient activity of glycosylasparaginase results in accumulation of undegraded aspartylglucosamine and other glycoasparagines in the body fluids and tissues of patients with AGU.[5] Developmental delays around 12–15 months of age are often the first neurologic signs of AGU.[1] Owing to the features of storage disorders, the disease progresses over the time span of years, leading to ID, autistic features, and abnormal skeletal and connective tissue growth with gait abnormalities.

In AGU, recurrent diarrhea is reported,[1] and the present patient also had persistent emesis and diarrhea, which led to the need for IV nutrition during his terminal stage. These conditions exacerbated the patient's existing malnutrition, and in turn, his susceptibility to various infections such as bacteremia and urinary and respiratory infections, which eventually became the cause of his death.

Low global awareness of AGU may result in a missed or delayed diagnosis because it often shows nonspecific findings such as developmental delay, including delayed speech or clumsy walking in early childhood. Sometimes patients may

Figure 3 Z-Score Plots of the Patient's and Control Urine Samples

N-linked Oligosaccharide	*z* score	Concentrations in urine (mmol / mol creatinine)		
	0 10^1 10^2 10^3	Patient	Mean	Std
334_aspartylglucos		971.59	1.09	1.54
496_aspartylglucos		178.92	0.14	0.18
787_aspartylglucos		499.18	0.92	1.31

N-linked Oligosaccharide	*z* score	Concentrations in urine (mmol / mol creatinine)		
	0 10^1 10^2 10^3	Control	Mean	Std
334_aspartylglucos		0.65	1.47	1.54
496_aspartylglucos		0.15	0.16	0.18
787_aspartylglucos		1.09	1.11	1.32

Elevated AGU-specific biomarkers (squares) are observed in the urine sample of the patient (upper table), but they are not detectable in the control urine sample (lower table). Pathologic AGU-specific reference values are indicated as dots. Semiquantitative concentrations of each AGU biomarker are indicated next to the Z-score plot (for additional details, see Hagemeijer et al., 2023[4]). 334_aspartylglucos = Asn-GlcNAc, 496_aspartylglucos = Asn-GlcNAc-Gal, and 787_aspartylglucos = Asn-GlcNAc-Gal-NANA.

initially be diagnosed with autism spectrum disorder. On the other hand, AGU also has some characteristic features that distinguish it. Skeletal and connective tissue abnormalities, as well as coarse facial features with a broad nasal bridge, are examples of such characteristic features. In addition, the imaging finding of T2 hypointensities in the pulvinar nuclei of the thalami has been reported.[6]

Although no fundamental cure for AGU has been established at this time, treatment options are being vigorously investigated.[1,7,8] Furthermore, very recently, branch points have been reported as a new therapeutic target for splicing abnormalities in genetic disorders.[9]

ID is defined as a developmental disability that includes deficits in both intellectual and adaptive functioning in conceptual, social, and practical domains. It is a relatively common condition that occurs in 1%–3% of the population. Severe ID (IQ < 50) is highly associated with biological and genetic causes. In children with severe ID, the most frequent cause is chromosomal abnormalities, with a rate of ~20%, while IMDs and neurodegenerative diseases account for ~7%.[10]

An excellent review of genetic testing in neurodevelopmental disorders was published by Savatt and Myers in 2021. In brief, first, physicians should complete the physical examination and collect the developmental, medical, and family histories. Then, if a specific etiology is suspected, appropriate specific genetic testing should be performed. Otherwise, cases should be copy number variant analyzed by chromosomal microarray (CMA) and/or exome sequencing (ES) performed by high-throughput sequencing technologies.[11] Current evidence suggests that among patients with ID or global developmental delay, the diagnostic yields are at least 15% for CMA and 35% for ES.[11]

Whereas genetic abnormalities in many IDs are difficult to treat at this time, IMDs constitute a subgroup of rare genetic conditions for which an increasing number of treatments have become available. The incidence of IMDs with ID is low, ranging from 1:10,000 to less than 1:200,000, their recognition is important because treatability outweighs the rare nature of these conditions.[12]

The following suggested diagnostic procedures for ID caused by IMDs can be drawn from the lessons learned from this case report.

First, ID caused by IMDs may be found on newborn screening of urine or blood. Newborn screening tests are conducted for the purpose of early detection and treatment of children with treatable diseases that, if left untreated, would cause intellectual or physical disabilities. Tandem mass spectrometry, which can detect abnormalities of amino acid metabolism, organic acid metabolism, and fatty acid metabolism, has also been introduced as part of newborn screening programs worldwide. The diseases covered by newborn screening vary by country or region considering the cost-effectiveness. AGU is included in newborn screening in Finland, where the disease frequency is high, but at present, as in Japan, it is not included in many countries.

Second, accurate diagnosis can be readily achieved if the physician is able to identify features suggestive of the causative disease of ID, such as the distinctive facial features and MRI findings of AGU. However, this means that, in areas where the prevalence of disease is not as high, it might result in missed diagnoses. It has additionally been reported that IMDs are suspected in cases of epilepsy with neurologic symptoms and regression.[13] In such situations, especially if lysosomal storage disorders are suspected, urinary analysis by UHPLC/HRAM mass spectrometry to screen for oligosaccharidoses is very useful.[4]

Finally, trio-WES is a powerful tool and has led to rapid identification of novel genes responsible for ID, with approximately 1,400 reported in a 2020 review.[14] In a large cohort study, 41.8% (1,796 of 4,293) of cases with severe undiagnosed developmental disorders had a pathogenic de novo variation.[15]

It is in fact desirable to identify the causative gene whenever possible because some lysosomal storage diseases, such as Gaucher disease, Fabry disease, mucopolysaccharidosis (MPS) types I, II, and VI, and Pompe disease, are already amenable to enzyme replacement therapy, and similar therapies can be expected to be developed for such diseases in the future.

Acknowledgment

The authors thank George Ruijter and David Vos (Department of Clinical Genetics, Erasmus University Medical Center Rotterdam, Netherlands) for suggestions and technical assistance and Hiroaki Ohara (Department of Neurology, Japanese Red Cross Wakayama Medical Center, Wakayama, Japan) for fruitful discussions.

Study Funding

The authors report no targeted funding.

Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NG for full disclosures.

Publication History

Received by *Neurology: Genetics* November 29, 2023. Accepted in final form April 8, 2024. Submitted and externally peer reviewed. The handling editor was Editor Stefan M. Pulst, MD, Dr med, FAAN.

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Continued

Appendix *(continued)*

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