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Case Report

Aspartylglucosaminuria caused by a novel homozygous mutation in the AGA gene was identified by an exome-first approach in a patient from Japan

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Abstract

Background: Aspartylglucosaminuria (AGU) is an autosomal recessive lysosomal storage disorder caused by a deficiency of the lysosomal enzyme, aspartylglucosaminidase (AGA). This disorder is rare in the general population except in Finland. Since the most characteristic feature of this disorder is a progressive developmental regression, patients often show no specific symptoms in the initial stages, and thus early diagnosis is often challenging.

Case report: We encountered a 16-year-old boy who began to show difficulties in his speech at the age of 6 years. Due to a mild regression in his development, he gradually lost common daily abilities. His diagnosis was first obtained through exome sequencing that identified a novel homozygous mutation in the *AGA* gene. This result was reasonable because of parental consanguinity. Reduced enzymatic activity of AGA was then confirmed. His urine was retrospectively screened by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and a specific pattern of abnormal metabolites was identified.

Conclusions: Because both exome sequencing and MALDI-TOF-MS screening are adaptable and comprehensive, future combinatory use of these methods would be useful for diagnosis of rare inborn errors of metabolism such as AGU.

Keywords: Aspartylglucosaminuria (AGU); Aspartylglucosaminidase (AGA); Lysosomal storage disorder; Developmental regression; Exome sequencing; Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS)

1. Introduction

Aspartylglucosaminuria (AGU: MIM #208400 referred to the Online Mendelian Inheritance in Man [http://omim.org/]) is an autosomal recessive lysosomal storage disorder caused by a deficiency of the lysosomal enzyme, aspartylglucosaminidase (AGA; EC 3.5.1.26). It was identified about 50 years ago [1]. In 1991, the *AGA* gene, responsible for AGU, was mapped to human chromosome 4q34.3 [2,3]. Although AGU is common in the Finnish population due to an existing common mutation within the population [4], it is rather

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infrequent in Japan, only one patient had been ever reported before the gene responsible was identified [5]. The main clinical feature is progressive developmental regression and there is no specific finding such as hepatosplenomegaly in early stage [6]. Thus, clinical diagnosis is often challenging in the initial stage. Final diagnosis requires biochemical studies such as enzymatic analysis; however, such specific tests are sometimes unavailable because of the disease rarity. Nevertheless, recent developments in comprehensive genetic and biochemical testing can overcome such difficulties.

In this study, a sporadic patient with developmental deterioration due to unknown etiology was diagnosed with AGU by exome sequencing.

2. Case report

A 16-year-old boy was born by normal delivery at 40 weeks of gestation. He is the fourth child of 5 siblings from first-cousin parents of Japanese origin. His birth weight was 3328 g (50–75th centile), height was 51.0 cm (75–90th centile), and occipitofrontal circumference (OFC) was 35.0 cm (75–90th centile). His early development was normal and he was admitted to an elementary school at the normal age. He then began to show difficulties in his speech at 6 years of age. When he was 12 years old, developmental regression became apparent, and his intelligence quotient evaluated by the Wechsler Intelligence Scale for Children-III was 40. At present, he shows poor reactions. He has lost common abilities in his daily life including toilet habits and maintaining hygiene. There was no episode of recurrent upper respiratory infection or recurrent otitis media, inguinal hernia, and seizures. His facial features are coarse, consisting of a big nose and thick lip vermilion (Fig. 1A). Currently, his height is 152.7 cm (<3rd centile), weight is 46.1 kg (3rd–10th centile), and OFC is 54.5 cm (<10th centile), indicating growth failure and progressive microcephaly. There was no hepatosplenomegaly. Brain magnetic resonance imaging (MRI) revealed white matter abnormalities (Fig. 1B, C).

Since the genetic cause of the patient's symptoms was unknown, exome sequencing was performed as the first step to screen single-nucleotide variants. Owing to the consanguineous family history, we focused on homozygous variants. Finally, the only candidate variant identified with this criteria was in exon 3 of *AGA*, NM_000027.3(AGA):c.386G>T (NP_000018.2:p.Gly129Val), which was checked visually using Integrative Genomics Viewer (IGV; http://www.broadinstitute.org/igv/) (Fig. 2A, B). The affected amino acid is conserved among species (Fig. 2C). The prediction scores suggested pathogenesis, i.e. SIFT (the name of program abbreviated from "Sorting Intolerant From Tolerant"; http://sift.jcvi.org/) (0; deleterious) and Polymorphism Phenotyping v2 (PolyPhen-2; http://genetics.bwh.harvard.edu/pph2/) (0.999; probably damaging). From these findings, this variant was considered to be a disease-causing mutation. Subsequent Sanger sequencing confirmed that this variant was inherited from both parents (Fig. 2D).

Finally, a fluorometric assay for plasma AGA activity was performed [7] and it was found to be 5.9 mU/L (control: 31.0 mU/L), indicating low activity of AGA. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) performed using AXIMA Performance (Shimadzu, Kyoto, Japan) showed abnormal peaks in the patient urine, i.e., 358.2, 520.2, 811.2, and 885.2 m/z (Fig. 3). These detected peaks were assigned as asparagine (Asn) associated aspartylglucosamine derivatives (Fig. 3).

3. Discussion

The patient showed a coarse face, which was one of the clues to suspect a lysosomal disorder [5]. Brain MRI also revealed characteristic findings, including decreased T2-signal intensity in the thalami and

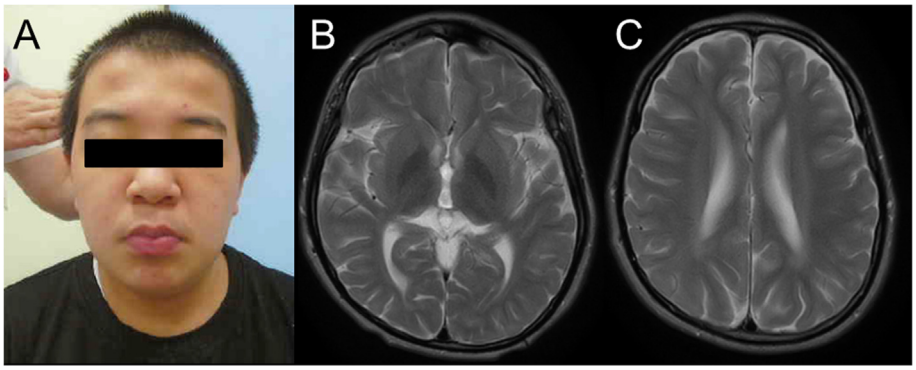

Fig. 1. Clinical information of the patient. (A) The patient showed coarse facial features with subcutaneous nodules. Written informed consent for use of this photo has been obtained from his parents. (B) Brain magnetic resonance imaging shows decreased T2-intensity in the thalami. Mild T2-high intensity is diffusely observed in the white matter.