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Original article

The first report of Japanese patients with asparagine synthetase deficiency

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Abstract

Background: Asparagine synthetase (ASNS) deficiency was recently discovered as a metabolic disorder of non-essential amino acids, and presents as severe progressive microcephaly, intellectual disorder, dyskinetic quadriplegia, and intractable seizures.

Methods: Two Japanese children with progressive microcephaly born to unrelated patients were analyzed by whole exome sequencing and novel *ASNS* mutations were identified. The effects of the *ASNS* mutations were analyzed by structural evaluation and *in silico* predictions.

Results: We describe the first known Japanese patients with ASNS deficiency. Their clinical manifestations were very similar to reported cases of ASNS deficiency. Progressive microcephaly was noted during the prenatal period in patient 1 but only after birth in patient 2. Both patients had novel *ASNS* mutations: patient 1 had p.L145S transmitted from his mother and p.L247W which was absent from his mother, while patient 2 carried p.V489D and p.W541Cfs*5, which were transmitted from his mother and father, respectively. Three of the four mutations were predicted to affect protein folding, and *in silico* analyses suggested that they would be pathogenic.

Conclusion: We report the first two Japanese patients with ASNS deficiency. Disease severity appears to vary among patients, as is the case for other non-essential amino acid metabolic disorders.

Keywords: Asparagine synthetase deficiency; Non-essential amino acid metabolic disorder; Microcephaly; Cerebral atrophy; Developmental delay; Intractable seizures

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1. Introduction

Asparagine synthetase (ASNS) deficiency is a rare autosomal recessive disease characterized by progressive microcephaly, cerebral atrophy, delayed myelination, and severe developmental delay. Other clinical features include intractable seizures, appendicular spasticity, difficulties with feeding, and sometimes respiratory insufficiency, which is the most life-threatening symptom. It is caused by homozygous or compound heterozygous mutation(s) in *ASNS* on chromosome 7q21, which encodes the enzyme asparagine synthetase [1]. ASNS converts aspartate to asparagine in the presence of glutamine and ATP [2].

Asns gene-trap mice brains were reported to have a reduced cortical thickness and an enlarged lateral ventricle area, suggesting that ASNS might be associated with the development and maturation of the human brain [1]. To date, six pathogenic *ASNS* variants have been reported [1,3–5] in patients with a wide range of ethnic origins, including Iranian Jews, Bangladeshi, French Canadian, Emirati, Saudi Arabian, and Chinese/Brunei mixed.

Here, we report the clinical presentations of two Japanese cases who showed clinical symptoms similar to reported cases and were revealed to have ASNS deficiency by whole exome sequencing (WES). To our knowledge, this is the first report of ASNS deficiency in the Japanese population.

2. Material and methods

2.1. Proton magnetic resonance spectroscopy (MRS)

Brain MRS of Patient 2 was performed at 8 months of age using a clinical 1.5T MR system (SIGNA twinspeed; GE Healthcare, Little Chalfont, UK) with a single-voxel acquisition. MRS was conducted using a point resolved spectroscopic localization sequence (PRESS), a water presaturation pulse sequence, and the following parameters: TE/TR: 30/2000 ms; number of excitations (NEX): 8; and number of points: 64. The volume of interest (VOI) was located in the basal ganglia and measured 15 × 15 × 15 mm (*x*, *y*, *z* axes). Brain MRS at 19 months was performed on a clinical 3T MR system (Quasar Dual; Philips Healthcare, Best, The Netherlands) with a single-voxel acquisition. MRS was performed using a PRESS, a water presaturation pulse sequence, and the following parameters: TE/TR: 31/5000 ms; NEX: 32; spectral bandwidth: 2000 Hz; and number of points: 1024. The VOI was located in the basal ganglia and measured 20 × 15 × 15 mm (*x*, *y*, *z* axes). All spectra of the same VOI were obtained without the water presaturation pulse sequence using NEX values of 2, and used to correct eddy current-induced phase shifts and to quantify brain metabolite concentrations. Pairs of MRS scans took ~5 min (scan time, 3 min 20 s) to obtain.

2.2. Whole exome sequencing

Genomic DNA was captured using the SureSelect Human All Exon v5 Kit (Agilent Technologies), and sequenced on HiSeq2500 (Illumina) with 101 bp paired-end reads. Exome data processing was performed as previously described [6]. We focused on rare nonsynonymous variants with minor allele frequencies below 1% in the dbSNP135 database; variants were not found in more than five of our in-house 575 control exomes. The inheritance of each mutation from different parents (compound heterozygotes) was verified using Sanger sequencing. Mutation damage prediction was made using Sorting Intolerant from Tolerant (SIFT; http://sift.jcvi.org), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2) and Mutation Taster software (http://www.mutationtaster.org).

2.3. Protein modeling

A homologous structure for human ASNS was searched for by the Protein Homology/analog Y Recognition Engine v2.0 (Phyre2) modeling server [7] using full-length human ASNS as a query. *In silico* predictions of free energy change following mutations were made using FoldX software version 3 beta [8]. FoldX calculations were repeated three times and presented as an average value with standard deviations.

2.4. Informed consent and ethical approval

All parents provided written informed consent. Experimental protocols were approved by the institutional review board of Yokohama City University School of Medicine.

3. Results

3.1. Case report

3.1.1. Patient 1

A 1-year-old boy with clinical manifestations of severe dyskinetic quadriplegia, epilepsy, microcephaly, and profound developmental delay was referred to Takuto Rehabilitation Center. He was born without asphyxia after 37 weeks of pregnancy by vaginal delivery, and has non-consanguineous Japanese parents. Microcephaly was noted by ultrasonography at a gestational age (GA) of 24 weeks. His birth weight, length, and head circumference (HC) were 2408 g (−1.4 standard deviation (SD)), 45.0 cm (−1.9 SD), and 29.0 cm (−3.0 SD), respectively. A lack of response to visual stimuli was noticed from birth. Feeding difficulties were