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Mutation-in-Brief

A Japanese boy with fructose-1,6-bisphosphatase deficiency who had a novel *FBP1* mutation (p.Phe90Val)

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Introduction

Fructose-1,6-bisphosphatase (FBPase) deficiency (OMIM 229700; FBPase; E.C.3.1.3.11) is a very rare autosomal recessive disorder of gluconeogenesis with a frequency of 1–9 per 100,000, which is characterized by recurrent episodes of hypoglycemia with metabolic and lactic acidosis, apnea, hyperventilation, and ketosis (1, 2). Fructose intake, fasting, and febrile infectious disease are known to trigger these symptoms. Once the diagnosis is established, the prognosis of this disorder is excellent if simple measures are followed such as the prevention of hypoglycemia (2) and avoidance of the consumption of foods with fructose (2) and glycerol (3).

FBPase deficiency can be definitively diagnosed by confirming mutations in *FBP1*, which encodes fructose-1,6-bisphosphatase-1. *FBP1* consists of 7 exons, which span more than 31 kb at chromosome 9q22.2-q22.3 and encodes a 362 amino acid protein that is mainly expressed in the liver and kidney. Since the first identified mutations in 1995, at least 36 additional mutations resulting in FBPase deficiency, including those from the Japanese population (4), have been described in the genomic region spanned by *FBP1*. Excretion of glycerol and glycerol-3-phosphate in the urine may help to distinguish this disease from other metabolic acidosis diseases (5). Here, we report a patient with FBPase deficiency caused by novel compound heterozygous mutations in *FBP1*, who had normal urine glycerol-3-phosphate during an oral fructose tolerance test.

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Patient Report

The patient was an 18-mo-old boy born to non-consanguineous healthy Japanese parents at full term after an uncomplicated pregnancy and delivery. He had no remarkable medical history during infancy.

He was admitted to our hospital at 18 mo of age with drowsiness without any obvious fructose intake. Physical examinations revealed a pale face, hepatomegaly, and Kussmaul respiration (respiration rate, 52/min). His body weight was 9.4 kg (–1.0 SD). FBPase deficiency was suspected because of a combination of lactic acidosis (pH, 7.135; serum bicarbonate level, 4.1 mmol/L; base excess, –22.4 mmol/L; plasma lactate level, 78.7 mg/dL) and hyperuricemia (serum uric acid level, 17.1 mg/dL). Hyponatremia (serum sodium level, 124 mEq/L) was also noted, which was probably a result of the vomiting and diarrhea. Other blood examination results included mild hypoglycemia (serum glucose level, 71 mg/dL), ketosis (serum total ketone body level, 5150 µmol/L; acetoacetate, 526 µmol/L; 3-hydroxybutyrate 4630 µmol/L), and increased levels of ammonia and pyruvate (plasma ammonia level, 112 µg/dL; serum pyruvic acid level, 2.01 mg/dL; lactate/pyruvate ratio, 39). Excretion of lactate and ketone bodies in his urine were also increased. Computed tomography scans of his abdomen revealed moderate hepatomegaly and a fatty liver.

After symptomatic and biochemical improvements with a glucose infusion (GIR 3.0 mg $kg^{-1}min^{-1}$) for one wk, an oral fructose tolerance test (1 g/kg) was performed. In this test, hypoglycemia (serum glucose levels decreased from 70 to 45 mg/dL) was noted, and lactate and uric acid levels were increased (lactic acid levels from 27.3 to 54.4 mg/dL, and uric acid levels from 4.4 to 8.9 mg/dL). Excretion of glycerol in the urine was markedly high at 293.8 $mmol^{-1}mol^{-1}cre$ (control: 38.1 ± 13.4 $mmol^{-1}mol^{-1}cre$), and excretion of glycerol-3-phosphate was normal (5.0 $mmol^{-1}mol^{-1}cre$). These levels were analyzed using gas chromatography-mass spectrometry (GC/MS) with a urease pretreatment non-extraction method. Taken together, these findings supported the diagnosis of FBPase deficiency, except for the glycerol-3-phosphate excretion levels in the urine.

Mutational Analysis

The study was approved by the Institutional Review Board of the Tokyo Metropolitan Children's Medical Center, and informed consent for the molecular study was obtained from the parents. Genomic DNA was extracted from the peripheral blood leukocytes of the patient and his parents. We used PCR-direct sequencing to examine all coding exons and flanking introns of *FBP1*. Direct sequencing of *FBP1* revealed compound heterozygous *FBP1* mutations (c.530C>A, p.Ala177Asp; and c.268T>G, p.Phe90Val) in the patient (Fig. 1A and B). His father carried the p.Ala177Asp mutation and his mother carried the p.Phe90Val mutation (Fig. 1A and B).

Previously, the p.Ala177Asp mutation was identified in a Japanese patient with FBPase deficiency. The pathogenicity of the Ala177Asp mutation in FBPase was verified with a functional assay; the enzymatic activity was markedly reduced (0.2 units/mg in mutant, 6.8 ± 0.5 units/mg in wild type) (2). The p.Phe90Val mutation was novel, was not detected in any of the 150 healthy controls tested, and was absent from various databases including dbSNP, the 1000 Genomes Project, Exome Variant Server, NHLBI Exome Sequencing Project, and the Human Genetic Variation Database in Japanese Population. *In silico* analyses with SIFT (http://sift.jcvi.org/) and M-CAP (http://bejerano.stanford.edu/mcap/index.html) predicted that the mutation would cause functional damage (SIFT score 0.02, M-CAP score 0.043).

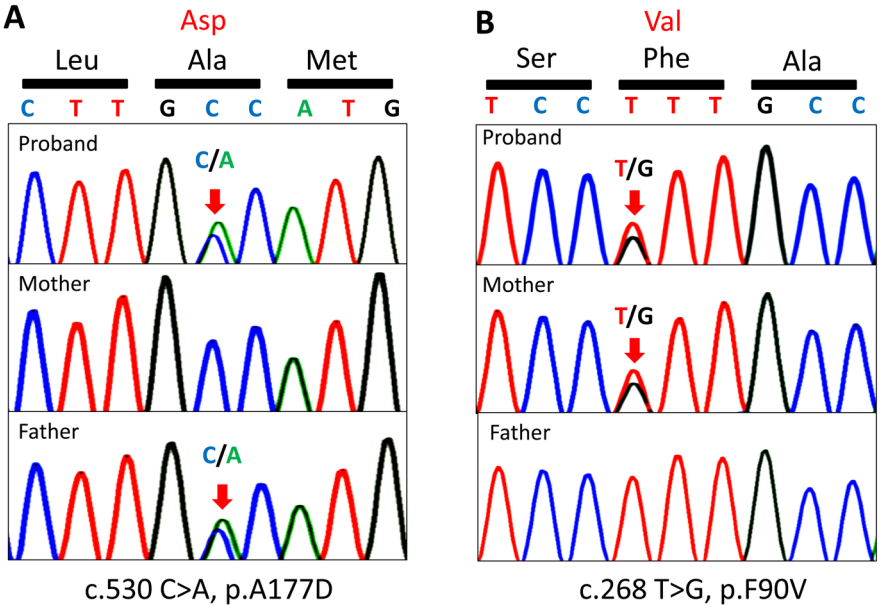

Fig. 1. Mutational analysis of *FBP1*. A: The chromatograms of the proband and the mother indicate a heterozygosity of aspartic acid [GAC] in place of alanine [GCC] at codon 530. The arrow indicates the mutated nucleotide. B: The chromatograms of the proband and the father indicate a heterozygosity of valine [GTT] in place of phenylalanine [TTT] at codon 268. The arrow indicates the mutated nucleotide.

Discussion

Here, we report a case of FBPase deficiency with compound heterozygous mutations, p.Phe90Val and p.Ala177Asp, in *FBP1*.

In general, urinary organic acid analysis using gas chromatography-mass spectroscopy (GC/MS) is very useful for screening FBPase deficiency (5). The fructose tolerance test from our patient showed a high level of glycerol excretion in the urine, whereas the excretion of glycerol-3-phosphate was at a normal level. The mechanism responsible for the normal concentration of glycerol-3-phosphate was not immediately clear. Kato *et al.* (2015) reported a case of FBPase deficiency in which the excretion level of glycerol-3-phosphate in the urine during a fasting episode was at a normal level based on GC/MS analysis after solvent extraction (6). However, excretion of glycerol-3-phosphate in the same sample was found to be increased when analyzed using GC/MS with the urease pretreatment non-extraction method (6). In our case, the normal value of glycerol-3-phosphate excretion in the urine was a false-negative, although the urine sample was analyzed using GC/MS with the urease pretreatment non-extraction method. Alternatively, a large amount of fructose during the oral tolerance test resulted in the excessive consumption of a derivatizing agent such that glycerol-3-phosphate in the urine was not well derivatized and could not be detected in the GC/MS analysis, thus causing the insufficient excretion of glycerol-3-phosphate.

FBPase deficiency is a fatal illness and is associated with a particularly high mortality rate during the neonatal period (3). Therefore, an early definitive diagnosis by genetic analysis is important for any suspected cases of this disease, which then eliminates the need to perform a potentially risky fructose tolerance test as was done in this case. Urgent treatment

of hypoglycemia and appropriate diet control can prevent sudden infant death and improve growth in patients with FBPase deficiency. In cases in which the first child is diagnosed with FBPase deficiency, genetic analysis of the parents is important for carrier detection to predict whether the siblings will be affected. In the present case, given that the parents were respective carriers for each of the detected mutations, genetic analysis of the next child may facilitate early diagnosis of FBPase deficiency before the onset of symptoms.

Conflict of Interest: The authors have nothing to declare.

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