

## Case Report

# Fructose 1,6-bisphosphatase deficiency: clinical manifestations and genetic features in two Chinese patients

Sanmei Wang, Jing Sun, Xiangyong Kong, Changshuan Yang, Zhichun Feng

*Department of Neurology Development, The Military General Hospital of Beijing PLA, Bayi Children's Hospital, 5 Nanmencang Hutong, Dongcheng District, Beijing 100700, China*

Received March 28, 2016; Accepted October 19, 2016; Epub April 15, 2017; Published April 30, 2017

**Abstract:** Fructose 1,6-bisphosphatase (FBPase) deficiency is a rare disease that is caused by mutations of FBP1 gene. We report our experience with two Chinese patients with FBPase deficiency. Two Chinese infants with FBPase deficiency presented with episodic hypoglycemia and acidosis occurring after weaning. In both patients, 960/961insG mutation of FBP1 gene was identified on genetic testing. Both patients readily responded to symptomatic treatment and appropriate dietary measures, and did not relapse. However, due to the delay in diagnosis, one patient developed disability resulting from severe brain damage. In conclusion: Early definitive diagnosis of FBPase deficiency is critical to its effective treatment, and is associated with an excellent prognosis. Same mutation in FBP1 gene could result in varied symptoms and genetic testing is a reliable method for early diagnosis of this very rare disease.

**Keywords:** Fructose 1,6-bisphosphatase deficiency, hypoglycemia, acidosis, genetics testing

## Introduction

Fructose-1,6-bisphosphatase (FBPase) deficiency is a very rare autosomal recessive disorder caused by a mutation in the fructose-1,6-bisphosphatase gene (FBP1), a key enzyme modulating the last steps of gluconeogenesis [1]. FBPase deficiency impairs the production of glucose from all gluconeogenic precursors including dietary fructose. The disease is characterized by hyperventilation, apnea, hypoglycemia, ketosis, and lactic acidosis. The condition is often life-threatening in the neonatal period and during infancy [2]. The symptoms are often triggered by febrile infections or fasting.

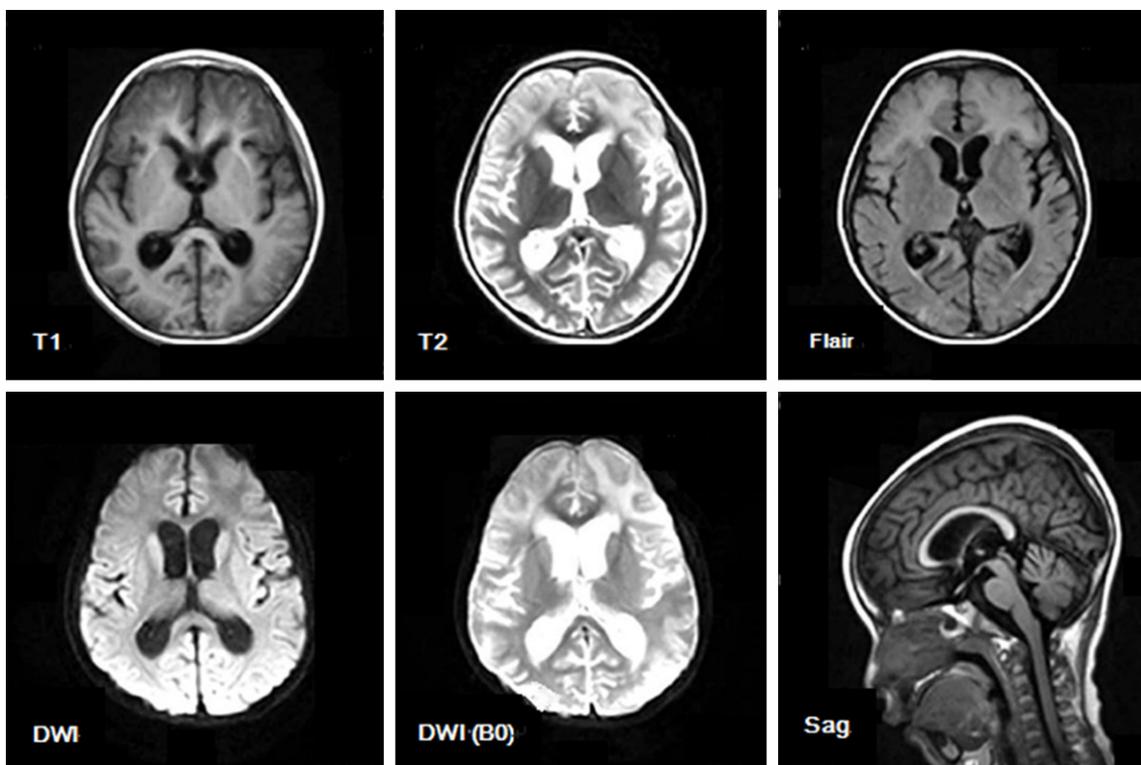
The enzyme FBPase is encoded by the FBP1 gene that is located on chromosome 9q22.3. In suspected cases, the diagnosis should be confirmed by genetics testing as early as possible [3]. Over 20 mutations in FBP1 gene leading to FBPase deficiency are documented [2, 4, 5]. The mutation of c.960insG appears to be more common in Japanese and German patients.

Ever since its first description in 1970 by Baker and Winegrad [6], only about 100 cases of FBPase deficiency have been documented in the published literature, with most cases being of Japanese descent [1, 7-10]. Occurrence of FBPase deficiency among the Chinese population is not well-characterized in the published literature. In this report, we describe our experience with two Chinese patients with FBPase deficiency who presented with metabolic disorders. A mutation (c.960insG) of FBP1 gene was identified in both patients by genomic sequencing.

## Case report

### Case 1

A female child (4.7 year old) was admitted at our hospital on January 8, 2013 with history of recurrent hypoglycemia and lactic acidosis since the last 3 years. The child was experiencing intermittent convulsions accompanied by altered sensorium since 1 month. She was borne of a full-term normal delivery and was



**Figure 1.** MRI examination at 3 months after onset of hypoglycemia accompanied by status epilepticus in the first patient. Diffuse brain parenchymal damage, more severe in the frontal lobe; ventricular enlargement, and sulcus deepening are observed. T1, T2, Diffusion weighted imaging (DWI), Flair, and Sag images showed diffuse demyelination in white matter, gray matter atrophy, and unclear junction of gray and white matter in bilateral frontal lobes. DWI showed high signals in bilateral basal ganglia that indicated a subacute lesion.

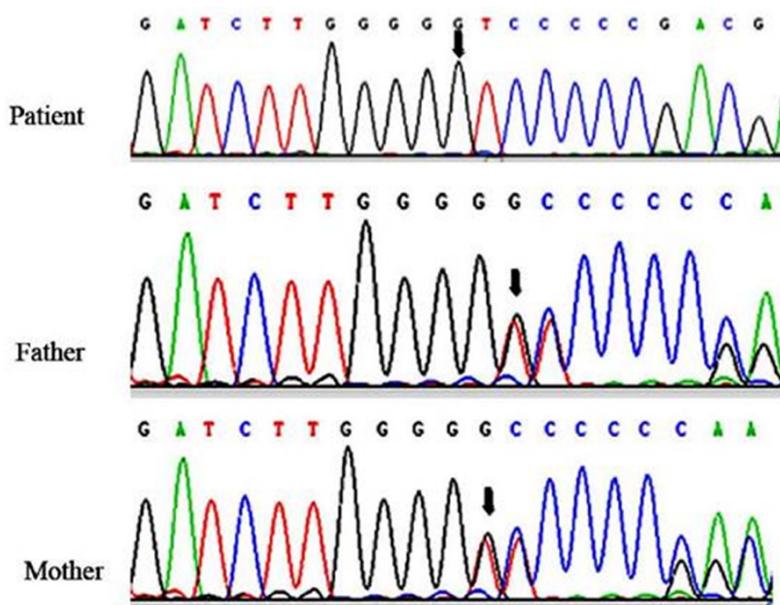
exclusively breast fed for 1 year and 4 months. Her parents were healthy and were not inter-married. There was no family history of genetic disease or specific toxic chemical contact. At the age of 1.5 years, she started with acute onset of excessive thirst in the morning, severe abdominal distension after drinking plenty of water, and recurrent vomiting, which appeared to have no obvious cause. She was hospitalized in a local hospital; laboratory investigations showed following results: blood glucose: 0.1 mmol/L, pH 6.91,  $\text{HCO}_3^-$  6 mmol/L,  $\text{Na}^+$  127 mmol/L, positive urine ketone (+++), negative urine glucose, normal hepatic and kidney function, and normal brain magnetic resonance imaging (MRI) findings. She recovered with symptomatic treatment that included glucose supplementation and correction of acidosis for 3 days. Thereafter, she had similar episodes (hypoglycemia and acidosis) that occurred two times each year (6 times in 3 years, of which one episode tended to occur in July each year). The episodes typically occurred at about 3:00

AM without any obvious cause, and were resolved after symptomatic treatment. During the episodes, she had good spirit and movement. At the age of 4.5 years, she experienced episodes of thirst, vomiting, abdominal distension, and lasting convulsions after trembling all over for about 4 hours. Although there was a remission in convulsions, she sustained severe brain injury, consciousness disorders, epilepsy, and hypermyotonia in limbs, after heavy dosage of sedatives and other treatment. The child was referred to our hospital for further investigation of the cause of her illness.

At admission, her vital signs were stable, with hypermyotonia in limbs, tendon hyperreflexia, and positivity in pathologic reflex. Laboratory examination of blood, urine, stool, and cerebrospinal fluid specimens were normal as were the hormone levels (blood insulin, pancreas hyperglycemia, cortical hormone, thyroid hormones, and growth hormone). Abdominal ultrasound examination did not reveal any abnormality.

**Table 1.** PCR primers for genetic testing of FBP1 gene

Exon	Forward primer (5'-3')	Reverse primer (5'-3')	Size of product (bp)
Exon1	GTGCTACTGCCCTCTCTTG	TTCTGGAGAGAGGGGCTACC	423
Exon2	CTTACCGAACACTCACTTTTGG	ATCTGCACAGTGGCTCAGG	440
Exon3	AAATCCCATTCTTAGCCCC	GCCACAGTGAATAGGACTGG	257
Exon4	CACCTTGAGAATGCCTCCTG	CACCTCCCACCTCCACATAC	331
Exon5	CACAGTCCCAGAGAGCCTG	AAGGATCCCTTTCATCTCCG	402
Exon6	CGGTGAATGGATGTGGAATC	CTAAACTAAATCGCGTGGGC	285
Exon7	TTGTCTCAGATGCAACCTGG	TAAGGTGCACAGCAGGTCTAG	455



**Figure 2.** Genetic testing on DNA identified 960/961insG mutation of FBP1 gene. The child was administered long-term antiepileptic treatment (oral oxcarbazepine 40 mg/kg/d). Dietary measures included avoidance of sucrose, fructose, and fruits with high sugar content, besides avoidance of fasting. During the follow-up period of 2.5 years, she did not suffer any episode of vomiting and hypoglycemia and manifested a good recovery in the chewing and swallowing function, with occasional twitching of mouth on the right side and hypermyotonia in limbs.

Urine gas chromatography-mass spectrometry (GC-MS) screening for inherited metabolic diseases revealed elevated levels of lactic acid, pyruvic acid, 4-hydroxy phenyl lactic acid, and 4-hydroxy benzoic acid. Digital video electroencephalograph (EEG) showed slightly slow background rhythm and frequent small spikes with low or medium amplitude in the left frontal, central and parietal area during the sleep phase. The head MRI suggested abnormal signals in the bilateral frontal, parietal and occipital lobe of the brain (Figure 1).

**Genetic testing:** Genomic DNA was isolated from peripheral white blood cells using the DNeasy minikit (QIAGEN®, German). The entire coding region of FBP1 gene was amplified for covering each exon by primers in Table 1. Polymerase chain reaction (PCR) amplification for each exon was performed for 35 cycles of 30 s at 94°C, 30 s at 60°C, 1 min at 72°C using 5 µL of 2.5 mM each dNTPs, 1 µL of 10 µM of each primer, 40 ng genomic DNA, 1 µL of 2.5 Unit TransStart-FastPfu DNA Polymerase and 10 µL of the supplied reaction buffer in final 50 µL of volume. PCR products were sequenced by an automated 3730XL DNA Sequencer (Applied Biosystems®, USA) and the results were analyzed with SeqScape 2.6 software (Applied Biosystems®). Molecular analysis showed homozygous 960/961 G insertion (designated “960/961insG”) in Core encoding sequence region, resulting in frame shift mutation after No. 32 amino acid sequence. Molecular analysis of DNA from both parents showed heterozygous mutation of 961 T/G mutation of FBP1 gene (Figure

2). Genetic testing confirmed the diagnosis of FBPase deficiency in this patient which presented with hypoglycemia and acidosis, and was accompanied by brain damage.

**Case 2**

A male infant (2 years old) was admitted at our hospital on July 2, 2014 with recurrent vomiting and poor thriving for six months, and one episode of hypoglycemic convulsions. He was borne of full term normal delivery. His grandparents were intermarried within three genera-

tions. He was exclusively breast fed after birth until the age of 1 year and 4 months. During this period, his intelligence and overall development was normal. Two months after stopping breast feeding, He gradually suffered recurrent vomiting, diarrhea, poor feeding, physical weakness, and poor thriving, apparently following mild cold symptoms. He recovered in about 3 days after intravenous glucose infusion and treatment of infection in a local hospital. Similar episodes occurred 5 times over a 7-month period with gradual aggravation of symptoms, including hematemesis after severe vomiting. He sustained general tonic clonic convulsions for about 5 minutes following by its remission. In the last 2 attacks, he manifested severe hypoglycemia and acidosis which, however, readily responded to symptomatic treatment.

Accessory examinations showed following results: Routine blood test: WBC ( $31.0 \times 10^9/L$ ), neutrophils (32%), polymorphonuclear neutrophil (55%), C-reactive protein (CRP) (4 mg/L); normal blood biochemistry and endocrine hormones; normal brain MRI; increased urinary lactate, pyruvate, and glycerol that was revealed by urine GC-MS screening, and elevated alanine level and lactic acidosis by blood tandem mass spectrophotometry (MS/MS). Genetic testing revealed the same mutation of FBP1 as in patient 1. Genetic testing of his parents could not be performed.

He was diagnosed as a case of FBPase deficiency based on his clinic manifestations and a mutation of FBP1 gene by molecular analysis. Similar dietary measures as that in the first patient were advised. During follow-up of 1.5 years, he was healthy and showed normal growth and development. He suffered from a febrile episode on two occasions, which did not trigger symptoms of hypoglycemia and acidosis.

### Discussion

In the present study, we report two Chinese patients with FBPase deficiency who presented with hypoglycemia and acidosis. Genetic testing revealed 960/961 del A ins G mutation of FBP1 gene in both patients. After symptomatic treatment and with appropriate dietary measures, their symptoms readily disappeared and did not relapse during the follow-up period.

FBPase deficiency is a very rare inborn error of gluconeogenesis, and was first identified in 1970 [1]. Till date, the number of reported cases in the world is still very limited and sporadic cases have been reported in different regions and ethnic populations. For example, 12 patients with FBPase deficiency were identified between 2001-2013 year in France, using molecular analysis and other diagnosis methods [2] and the incidence of FBP1 deficiency in the French population was estimated less than 1:900,000. FBP1 gene is mapped on chromosome 9q22.3 and encodes the enzyme FBPase, a key enzyme modulating the last steps of gluconeogenesis [1]. FBP1 deficiency is an inborn autosomal recessive disorder caused by a mutation in FBP1, resulting in impairment of glucose production from all gluconeogenic precursors including dietary fructose. This deficiency manifests clinically as hyperventilation, apnea, hypoglycemia, ketosis, and lactic acidosis, and is often life-threatening in the newborn period and infancy [2]. Generally, patients with FBPase deficiency are admitted to hospital due symptoms related to lactic acidosis and ketotic hypoglycemia, which are often triggered by fasting or febrile infections [11]. In these patients, hyperketonemia, an increase in the ratio of lactate and pyruvate, hyperalaninemia, glyceroluria, an increased uric acid level, and pseudo-hypertriglyceridemia are often found on laboratory examination [11, 12]. Although these laboratory findings are helpful for diagnosing FBPase deficiency in suspected cases, the mutation of FBP1 gene by sequencing exons and the junction of intron-exon should be performed to confirm the diagnosis as early as possible [3]. Up to now, a total of 35 FBP1 mutations have been reported in published literature worldwide [13]. Among all FBP1 gene mutations, the mutation of c.960insG appears to be more common in Japanese, and also been founded in Europe and North American population [2, 5]. It is believed that more novel FBP1 gene mutations will be identified in further studies. Recently, a novel deletion mutation of a 5412 bp across exon 2 whole coding sequence in FBP1 gene were repeatedly identified in Turkey and American [13].

Generally, the first symptoms in FBPase deficiency are often triggered following an infectious disease or low food intake, with most cases presenting in early infancy [2]. In present

## FBPase deficiency in two Chinese patients

study, the two patients were under 5-years of age and had similar clinic manifestations including hypoglycemia and acidosis as reported in earlier reports of FBPase deficiency [12, 14, 15]. The diagnosis was confirmed by a relatively common 960/961insG mutation in FBP1 gene [16, 17]. Both parents of the first patient were heterozygous mutation carriers, resulting in a probability of homozygous 960/961insG mutation in the patient. The parents of the second patient did not undergo genetic testing for FBP1 mutation; however, we speculate that his parents, too, were carriers of this mutation, since his grandparents were genetically related.

The two patients had some similarities in clinical presentation. Both patients were normal in the early infancy. After weaning, they gradually started with episodes of hypoglycemia and acidosis. In addition, the parents of the two patients, apparently, had an aversion for sweet fruits, likely due to the heterozygous mutation of 960/961insG in the FBP1 gene. However, on close scrutiny of their clinical course, the two patients manifested some distinct clinic characteristics from each other. First patient sustained six recurrent attacks over a period of 3 years, with no obvious triggering factor such as infection and fasting. We believe that the reason for the observed tendency of symptom occurrence in the month of July every year was because of the abundant availability of fruit in China during this period and that his occasional intake of fruit may have been the source of fructose intake. During the early phase of his illness, the child apparently had a normal brain development and did not show any abnormality on brain MRI scanning. With recurrent episodes of hypoglycemia and acidosis due to the delay of definitive diagnosis, general tonic clonic seizures appeared to have caused brain damage, resulting in the subsequent occurrence of disability. With appropriate dietary measures he did not suffer from similar attacks during the follow-up period of 2.5 years.

The second patient presented with recurrent diarrhea, vomiting, drowsiness, and convulsions after weaning, and a definitive diagnosis of FBPase deficiency was established soon after onset. His symptoms did not relapse and his development was normal well as observed on follow-up examination at 1.5 years. Our experience suggests that early definitive diag-

nosis of FBPase deficiency is critical, because once the diagnosis is confirmed, the treatment of FBPase deficiency is simple with an excellent prognosis.

Besides FBPase activity in monocytes or leukocytes [14, 15], genetic testing for mutation of FBP1 gene is a reliable method for early diagnosis of FBPase deficiency [16]. Thus detection of mutation in FBP1 gene should be performed using genetic testing to rule out the possibility of FBPase deficiency once metabolic related diseases are first suspected in infancy or early childhood, as this very rare disease is likely to be misdiagnosed or diagnosed at a later stage.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Zhichun Feng, Department of Neurology Development, The Military General Hospital of Beijing PLA, Bayi Children's Hospital, 5 Nanmencang Hutong, Dongcheng District, Beijing 100700, China. Tel: +8601066721786; Fax: +86-01066721786; E-mail: zhjfengzc@126.com

### References

- [1] Moon S, Kim JH, Han JH, Ko SH, Ahn YB, Kim JH, Yang SH and Song KH. Novel compound heterozygous mutations in the fructose-1,6-bisphosphatase gene cause hypoglycemia and lactic acidosis. *Metabolism* 2011; 60: 107-113.
- [2] Lebigot E, Brassier A, Zater M, Imanci D, Feillet F, Therond P, de Lonlay P and Boutron A. Fructose 1,6-bisphosphatase deficiency: clinical, biochemical and genetic features in French patients. *J Inher Metab Dis* 2015; 38: 881-887.
- [3] el-Maghrabi MR, Lange AJ, Jiang W, Yamagata K, Stoffel M, Takeda J, Fernald AA, Le Beau MM, Bell GI, Baker L, et al. Human fructose-1,6-bisphosphatase gene (FBP1): exon-intron organization, localization to chromosome bands 9q22.2-q22.3, and mutation screening in subjects with fructose-1,6-bisphosphatase deficiency. *Genomics* 1995; 27: 520-525.
- [4] Kikawa Y, Inuzuka M, Jin BY, Kaji S, Yamamoto Y, Shigematsu Y, Nakai A, Taketo A, Ohura T, Mikami H, et al. Identification of a genetic mutation in a family with fructose-1,6-bisphosphatase deficiency. *Biochem Biophys Res Commun* 1995; 210: 797-804.
- [5] Herzog B, Morris AA, Saunders C and Eschrich K. Mutation spectrum in patients with fruc-

## FBPase deficiency in two Chinese patients

- tose-1,6-bisphosphatase deficiency. *J Inherit Metab Dis* 2001; 24: 87-88.
- [6] Baker L and Winegrad AI. Fasting hypoglycaemia and metabolic acidosis associated with deficiency of hepatic fructose-1,6-diphosphatase activity. *Lancet* 1970; 2: 13-16.
- [7] Kikawa Y, Inuzuka M, Jin BY, Kaji S, Koga J, Yamamoto Y, Fujisawa K, Hata I, Nakai A, Shigematsu Y, Mizunuma H, Taketo A, Mayumi M and Sudo M. Identification of genetic mutations in Japanese patients with fructose-1,6-bisphosphatase deficiency. *Am J Hum Genet* 1997; 61: 852-861.
- [8] Wilcken DE and Wilcken B. The natural history of vascular disease in homocystinuria and the effects of treatment. *J Inherit Metab Dis* 1997; 20: 295-300.
- [9] Herzog B, Wendel U, Morris AA and Eschrich K. Novel mutations in patients with fructose-1,6-bisphosphatase deficiency. *J Inherit Metab Dis* 1999; 22: 132-138.
- [10] Faiyaz-UI-Haque M, Al-Owain M, Al-Dayel F, Al-Hassnan Z, Al-Zaidan H, Rahbeeni Z, Al-Sayed M, Balobaid A, Cluntun A, Toulimat M, Abalkhail H, Peltekova I and Zaidi SH. Novel FBP1 gene mutations in Arab patients with fructose-1,6-bisphosphatase deficiency. *Eur J Pediatr* 2009; 168: 1467-1471.
- [11] Steinmann B and Santer R. Disorders of fructose metabolism[M]//inborn metabolic diseases. Springer Berlin Heidelberg 2012; 157-165.
- [12] Afroze B, Yunus Z, Steinmann B and Santer R. Transient pseudo-hypertriglyceridemia: a useful biochemical marker of fructose-1,6-bisphosphatase deficiency. *Eur J Pediatr* 2013; 172: 1249-1253.
- [13] Santer R, du Moulin M, Shahinyan T, Vater I, Maier E, Muntau AC and Steinmann B. A summary of molecular genetic findings in fructose-1,6-bisphosphatase deficiency with a focus on a common long-range deletion and the role of MLPA analysis. *Orphanet J Rare Dis* 2016; 11: 44.
- [14] Ito M, Kuroda Y, Kobashi H, Watanabe T, Takeda E, Toshima K and Miyao M. Detection of heterozygotes for fructose 1,6-diphosphatase deficiency by measuring fructose 1,6-diphosphatase activity in their cultured peripheral lymphocytes. *Clin Chim Acta* 1984; 141: 27-32.
- [15] Kikawa Y, Takano T, Nakai A, Shigematsu Y and Sudo M. Detection of heterozygotes for fructose-1,6-diphosphatase deficiency by measuring fructose-1,6-diphosphatase activity in monocytes cultured with calcitriol. *Clin Chim Acta* 1993; 215: 81-88.
- [16] Beatty ME, Zhang YH, McCabe ER and Steiner RD. Fructose-1,6-diphosphatase deficiency and glyceroluria: one possible etiology for GIS. *Mol Genet Metab* 2000; 69: 338-340.
- [17] Eren E, Edgunlu T, Abuhandan M and Yetkin I. Novel fructose-1,6-bisphosphatase gene mutation in two siblings. *DNA Cell Biol* 2013; 32: 635-639.