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ORIGINAL ARTICLE

Exon 2 deletion represents a common mutation in Turkish patients with fructose-1,6-bisphosphatase deficiency

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Abstract

Fructose-1,6-bisphosphatase (FBPase) deficiency is an autosomal recessive inborn error of gluconeogenesis. We aimed to investigate clinical and biochemical findings and molecular genetic data in ten Turkish patients with fructose-1,6-bisphosphatase deficiency. Ten Turkish patients who were diagnosed with fructose-1,6-biphosphatase deficiency in a single center from 2013 to 2019 were included in this study. Their clinical and laboratory data were collected retrospectively. All patients were hospitalised in intensive care unit mostly after catabolic stress conditions such as infections, starvation and rarely fructose consumption. Prognosis was good after correct diagnosis and treatment. Molecular analyses of *FBP1* gene revealed a homozygous exon 2 deletion in eight patients, a novel homozygous c.910_911dupTT mutation in one patient and a homozygous IVS5 + 1G > A splicing mutation in one patient. Exon 2 deletion (previously termed exon 1) was found to be the most common mutation in Turkish fructose-1,6-biphosphatase deficiency patients.

Keywords Fructose-1,6-bisphosphatase deficiency · FBP1 gene · Fructose · Hypoglycemia · Coma · Lactic acidosis

Introduction

Fructose-1,6-bisphosphatase (FBPase) deficiency (OMIM#229700) is a rare autosomal recessive inherited disorder of fructose metabolism affecting gluconeogenesis (Steinmann and Santer 2016). It was first described in 1970 (Baker and Winegrad 1970) and responsible gene was found in 1995 (El-Maghrabi et al. 1995). It is encoded by the *FBP1* gene, located at 9q22.2-q22.3 and consists of eight exons, of which seven (Baker and Winegrad 1970; Bhai et al. 2018; el-Maghrabi et al. 1995; Eren et al. 2013; Erol et al. 2014; Faiyaz-Ul-Haque et al. 2009; Herzog et al. 2001) encode 338 amino acids, and spans over 31 kb. Its incidence is estimated as 1:350.000–1:900.000 in the Dutch and French population respectively and prevalence was reported as 1:1.782.321 in Southern Brazil (Visser et al. 2004; Lebigot et al. 2015; Pinheiro et al. 2019). Patients are generally well except in acute attacks which are triggered by catabolic stress such as prolonged fasting, often precipated by intercurrent infections or ingestion of large amounts of fructose. Abdominal pain, vomiting, irritability, hypervenitilation, apnea, somnolescence, coma, seizures, ketotic hypoglycemia, metabolic and lactic acidosis, hepatomegaly, hypertransaminasemia, hypertriglyceridemia, hyperuricemia are some of clinical and laboratory findings in catabolic stress. Increased plasma and urine alanine, lactate, glycerol, glycerol-3-phosphate and ketone bodies can be seen in acute attacks (Kato et al. 2015). Prognosis is well if the diagnosis is established early and with avoidance of fasting and infections.

In this present study we reviewed our FBPase deficiency patients retrospectively to investigate genotype-phenotype correlation in ten Turkish patients.

Material and methods

All patients diagnosed with FBPase deficiency by clinical, biochemical and molecular analyses from 2013 to 2019 in our single center were included in this study. Their clinical and laboratory data were collected retrospectively. Serum blood glucose, uric acid, liver enzymes, lipid profile, venous blood gases, lactate, pyruvate, blood amino acids, carnitine

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and acylcarnitine, urine organic acid and molecular genetic analyses were examined retrospectively. Molecular analysis of the *FBP1* gene was performed on genomic DNA by conventional Sanger sequencing and multiple-ligation probe amplification (MLPA). NM_001127628.1 was used as the reference sequence. No PCR product could be generated for exon 2 of the *FBP1* gene was called exon 2 deletion according to Santer et al. 2016. Sanger sequencing using specific primers that allow sequencing of a junction fragment was going on for this large deletion.

Ethical approval

The study protocol was approved by the Local Medical Research Ethics Committee of Kecioren Training and Research Hospital and conducted in accordance with the declaration of Helsinki.

Results

There were ten patients diagnosed with FBPase deficiency from nine unrelated families. The main clinical, biochemical and molecular findings are summarized in Table 1. Male to female ratio was 5/5. Consanguinity rate was 8/9 (89%). Age at first hospital admission was between 1 and 18 months and first symptoms were vomiting, irritability, frequent and deep breathing. Nine of ten patients (90%) presented after 6 months of age while one patient (10%) was symptomatic in the first day of life. The age at diagnosis ranged from 9 to 97 months. Multiple hospitalisations for recurrent hypoglycemia and ketoacidosis were seen in all patients. They were hospitalised in intensive care unit mostly after catabolic stress conditions such as infection (especially recurrent upper respiratory tract infections or sometimes acute gastroenteritis), prolonged fasting and rarely excessive fructose consumption. Symptoms at hospital admissions were vomiting, abdominal pain, irritability, hyperventilation, apnea, depressed consciousness and seizures. In physical examination somnolence, coma, Kussmaul breathing, hepatomegaly were determined in most patients during acute attacks. Laboratory findings showed hypoglycemia, metabolic acidosis, ketonuria, hyperuricemia, hypertransaminasemia, hypertriglyceridemia, hyperalaninemia and elevated lactate/pyruvate ratio. Urine samples were collected during acute crises in nine patients, six of which were shown to excrete glycerol. Hypertriglyceridemia was seen in 5/5 patients measured in acute crisis. Serum uric acid levels were increased in 8/8 patients measured in acute crisis. Serum lactate, pyruvate and lactate/pyruvate ratios were mostly increased in acute crises. Abdominal sonography showed mild to moderate hepatomegaly and mildly increased echogenicity of liver in the majority of patients. In urine organic acid analyses, glycerol, lactate and ketones were seen. Molecular analyses of *FBP1* gene revealed a homozygous gross deletion including exon 2 in eight patients (78%), a novel homozygous c.910_911dupTT mutation in one patient (11%) and a homozygous IVS5 + 1G > A splicing mutation in one patient (11%). Fructose, sucrose and sorbitol-restricted diet, frequent feeding and uncooked cornstarch (1.5–2 g/kg/day) before sleeping at night were offered with good clinical response. All patients are alive with normal growth and cognitive development at the time of writing.

Discussion

Incidence of FBPase deficiency is not known in Turkish patients. Because of higher rate of consanguinity, it might be more frequent than that estimated in European and other countries. All patients with FBPase deficiency had multiple episodic attacks triggered by infections, long-term fasting or rarely plenty of a load of fructose. One patient (10%) had first episode of hypoglycemia during the neonatal period, while the remaining nine patients (90%) were symptomatic between 6 months to 2 years of age. Although nearly half of FBPase deficiency patients are reported to present in the first 1 to 4 days of life secondary to glycogen depletion, only one (10%) patient was the symptomatic in the newborn period in our study (Steinmann and Santer 2016). All our patients, except one, were diagnosed after the first year of life. Patients mostly had a diagnostic delay ranging from months to years. The causes of delayed diagnosis were low awareness of this rare disease, little clinical experience and delayed consultation to a metabolic specialist. Hypoglycemia, metabolic acidosis, ketonuria, hyperuricemia, hypertransaminasemia, hypertrygliceridemia, hyperalaninemia and elevated lactate/pyruvate ratio and glycerol were observed in acute attacks in most of our patients in parallel to the published literature. All these parameters were normal while the patients were well. Hereditary fructose intolerance (HFI); other gluconeogenesis defects such as pyruvate carboxylase deficiency, phosphoenolpyruvate carboxykinase deficiency, glycogen storage disease type Ia-b; congenital lactic acidosis, organic acidurias and disorders of ketone utilization might be considered in the differential diagnosis of FBPase deficiency. Treatment with high glucose infusion rates with bicarbonate was useful in acute attacks in FBPase deficiency, organic acidurias and disorders of ketone utilization. An important clue for differential diagnosis is that lactate levels decrease with high glucose infusion rate in patients with gluconeogenesis defects and organic acidurias while they increase in patients with congenital lactic acidosis. Lactate level is normal in disorders of ketone utilization even in acute attacks. After treatment, normalization of triglycerides is an important clue for FBPase deficiency since their concentration remains elevated in GSD-I. Glycogen storage disorder type I, also had ‘dolls like face’, a

Table 1 Clinical, biochemical and molecular genetic findings of patients with fructose-1,6-bisphosphatase deficiency

Patient No ($N = 6$)	Sex	Age at onset/ admission1/2/ diagnosis/ current (month)	Consanguinity	Clinical findings at diagnosis	Glycerol (urine)	Hypertransaminasemia/ hyperuricemia/ hypertriglyceridemia	Lactate/ Pyruvate = ratio	Number of further episodes requiring hospital admission	Prognosis	Mutation in *FBP1* gene (homozygous)
1	F	6/6/30/31/91	+	Vomiting, hyperventilation, hypoglycemia, metabolic and lactic acidosis, ketonuria, hepatomegaly, grade 1 hepatosteatosis, recurrent hospital isation because of infections or consumption a lot of dessert, fruit or chocolate	–	+/+/NA	24/2.4 = 10* 23.6/2.1 = 11.2* 50.5/2.2 = 23 42.1/0.87 = 48.4*	multiple	well	IVS5 + 1 G > A splicing
2	F	12/13/13/14/60	+	Vomiting, hyperventilation, hypoglycemia, metabolic and lactic acidosis, ketonuria, hypercalcemia, hepatomegaly, grade 1 hepatosteatosis, right renal milimetric stone, left hepatic lateral segment hypoplasia, left portal vein thrombosis, history of exchange transfusion for neonatal jaundice, recurrent hospital isation because of infections	+	+/+/+	87.9/NA =? 76.2/1.7 = 44.8* 130.3/NA =?	multiple	well	Exon 2 deletion
3	M	7/7/12/13/44	+	Vomiting, hyperventilation,hypoglycemia, metabolic and lactic acidosis, ketonuria, mild hepatomegaly, grade 1 hepatosteatosis, recurrent hospital isation because of infections	+	+/+/–*	75.6/0.59 = 128.1 18.1/0.3 = 60.3*	multiple	well	Exon 2 deletion
4	M	1/1/70/71/97	- (same village)	Vomiting, hyperventilation, hypoglycemia, metabolic and lactic acidosis, ketonuria, recurrent hospital isation because of infections, recurrent hypoglycemic seizures, refusing desserts, honey and fruits	-*	+/–*/NA	66.7/0.97 = 68.8*	multiple	well	Exon 2 deletion
5	M	18/18/94/97/110	+	Vomiting, hyperventilation, hypoglycemia, metabolic and lactic acidosis, ketonuria, grade 1 hepatosteatosis, recurrent hospital isation because of infections	+	+/+/+	16.1/NA =?* 15.2/NA =?* 164.4/1.2 = 137	multiple	well	Exon 2 deletion
6	F	7/7/26/27/37	+	Vomiting, hyperventilation,hypoglycemia, metabolic and lactic acidosis, ketonuria, bilateral hypermetropia, strabismus, recurrent hospital isation because of infections	+	+/–*/–*	43.4/0.66 = 65.8*	multiple	well	Exon 2 deletion
[a]7	F	13/13/14/14/78	+	Vomiting, hyperventilation,hypoglycemia, metabolic and lactic acidosis, ketonuria, grade 1 hepatosteatosis, bilateraly developmental hip dysplasia, recurrent hospital isation because of infections	–	+/+/+	58.9/3.4 = 17.3	multiple	well	Exon 2 deletion
[a]8	F	14/14/14/14/61	+	Vomiting, hyperventilation,hypoglycemia, metabolic and lactic acidosis, ketonuria, bilateraly developmental hip dysplasia, recurrent hospital isation because of infections	–	+/± *	52.2/1.4 = 37.2	multiple	well	Exon 2 deletion
9	M	8/8/8/9/60	+	Vomiting, hyperventilation,hypoglycemia, metabolic and lactic acidosis, ketonuria, hepatomegaly, grade 1 hepatosteatosis, recurrent hospital isation because of infections	+	+/+/+	21.6/NA =?	multiple	well	**c.910_911dupTT**
10	M	12/12/12/13/140	+	Vomiting, hyperventilation,hypoglycemia, metabolic and lactic acidosis, ketonuria, hypophosphatemia, hepatomegaly, grade 1 hepatosteatosis, recurrent hospital isation because of infections	+	+/+/+	56/1.9 = 29. 5	multiple	well	Exon 2 deletion

[a] sibling, +: yes, −: no, NA: not available, *: sample was not taken in acute attacks, M: male, F: female, multiple: >3 attacks, (lactate: N: 4.5–20 mg/dl, pyruvate: N: 0.3–1 mg/dl), admission 1: another hospital, admission 2: our hospital at diagnosis, exon 2 deletion: 5412-bp deletion [c.-24-26_170 + 5192del (p.0?)] according to Santer et al. 2016, a novel mutation is designated in bold

large abdomen and thin arms and legs. In contrast to HFI, there is no aversion to sweet foods in patients with FBPase deficiency. Regular feeding with fructose-sucrose restriction and nightly cornstarch (1–2 g/kg/doses) allowed normal mental and motor development in all our patients (Table 1).

Nearly 50 mutations in the *FBP1* gene have been reported (Kikawa et al. 1995, 1997; Herzog et al. 2001; Faiyaz-Ul-Haque et al. 2009; Afroze et al. 2013; Eren et al. 2013; Lebigot et al. 2015; Santer et al. 2016; Li et al. 2017; Moey et al. 2018; Pinheiro et al. 2019). There are some population specific mutations such as c.958G > A (p.Gly320Arg), c.986 T > C (p.Leu329Pro), which are the most frequent pathogenic variants of *FBP1* in Southern Brazil; c.472C > T (p.Arg158Trp) and c.841G > A (p.Glu281Lys) in Pakistan and Indian subcontinent; c.959dupG (pSer321Valfs*13) in the Asian and Japanese populations; complete deletion of the *FBP1* gene (p.0?) in Germany; a gross deletion including exon 2 (5412-bp deletion) [c.-24-26_170 + 5192del (p.0?)] in Turkish and Armenian populations; c.841G > T (p.Glu281Ter) and c.114_119dupCTGCAC (p.Cys39_Thr40dup) in Arab ethnicity (Kikawa et al. 1997; Herzog et al. 2001; Faiyaz-Ul-Haque et al. 2009; Afroze et al. 2013; Santer et al. 2016; Ijaz et al. 2017; Bhai et al. 2018; Li et al. 2017; Pinheiro et al. 2019). Nearly 10–20% of reported patients were of Turkish origin (Santer et al. 2016). Previously, homozygous c.-24-26_170 + 5192del (p.0?), c.35delA (p.Asn12Thrfs*2), c.359C > T (p.Pro120Leu), c.616_619delAAAG (p.Lys206Valfs*70), c.660delT (p.Phe220Leufs*57), c.731_738delins20 (p.Arg244_Tyr245delins6), c.841G > A (p.Glu281Lys) and heterozygous c.881G > A (p.Gly294Glu) mutations are described in Turkish patients (Herzog et al. 2001; Eren et al. 2013; Lebigot et al. 2015; Bulut et al. 2015; Santer et al. 2016). There were also a few clinical case reports in Turkish patients without molecular analyses in the literature (Tavil and Sipahi 2003; Paksu et al. 2011; Erol et al. 2014; Gokcay et al. 2015). Interestingly exon 2 deletion (previously termed exon 1) was defined as founder mutation in Armenian and Turkish patients (Santer et al. 2016). Since it is a common mutation, PCR and MLPA (multiplex ligation-dependent probe amplification analyses) are the most useful techniques to determine this deletion.

Exon 2 deletion was found to be the most common mutation in Turkish patients with fructose-1,6-biphosphatase deficiency. FBPase deficiency should be considered in children with recurrent hypoglycemia, metabolic acidosis and ketonuria.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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