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Novel Fructose-1,6-bisphosphatase Gene Mutation in Two Siblings

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Fructose-1,6-bisphosphatase (FBPase) deficiency is an autosomal, recessively inherited disease that progresses with severe hypoglycemia, and metabolic attacks result in a defect in gluconeogenesis. If not appropriately treated, and if fructose is not excluded from the diet, the outcome could be fatal. Two Turkish children with FBPase deficiency were diagnosed based on mutation of the FBP1 gene. The first, a 2-year-old girl, was referred to our clinic because of lactic acidosis, uncorrectable hypoglycemia, and increased transaminases. FBPase deficiency was suspected in the patient, who recovered dramatically after a high-dose glucose infusion and adequate bicarbonate replacement. The second patient, a five-and-a-half-year-old male sibling of the patient, was also hospitalized, twice, because of hypoglycemic attacks and metabolic acidosis. Different from previous analyses, a homozygous c.658delT mutation was detected at exon 5 of the FBP1 gene in the two siblings. As a result of this mutation, there was a TGA (stop codon) at exon 6. There was first-degree consanguinity between the parents. These two cases were the first FBP1 gene mutations reported in our country.

Introduction

Fructose-1,6-bisphosphatase (FBPase) is a key enzyme in gluconeogenesis, and if there is a deficiency, prominent hypoglycemia and metabolic acidosis can develop. FBPase converts fructose 1,6 bisphosphate to fructose 6-phosphate and inorganic phosphate. The enzyme is most active in the intestine and liver (Steinmann *et al.*, 2001). FBPase deficiency is caused by FBP1 gene mutation. FBPase coded in two distinct genes which are called FBP1, FBP2. In muscle FBPase is coding by FBP2 gene (Asberg *et al.*, 2010). When liver glycogen stores are depleted, the body uses gluconeogenetic precursors, such as lactate, glycerol, and pyruvate, to maintain blood glucose levels. During periods of low food intake or infection, an FBP1 defect can result in hypoglycemia, ketonuria, elevated blood lactate, and metabolic acidosis. The deficiency can be fatal in neonates, but tolerance to fasting generally improves with age. Relatively few cases of FBPase deficiency have been described, and it has been suggested that patients with deficient FBPase have been misdiagnosed with sudden infant death syndrome or Reye's syndrome (Emery *et al.*, 1988; Zammarchi *et al.*, 1995). If this autosomal, recessively inherited disease is not diagnosed and treated early, it could result in a fatal outcome (Baker and Winegrad, 1970; Baerlocher *et al.*, 1971). In treatment, the main principle is to decrease fructose intake and to abstain from long-term fasting. Herein we present the clinical and genetic results of the cases of two siblings diagnosed with FBPase deficiency that progressed with hypoglycemia and metabolic acidosis.

Materials and Methods

Patient reports

Patient 1. A 2-year-old girl was referred to the emergency unit because of recurrent vomiting and hypoglycemia attacks. There were no drugs or chronic disease in the medical history; however, the information showed that the patient had been hospitalized 1 year prior with the same signs and symptoms. In addition, there was first-degree consanguinity between the parents. The physical examination at admittance revealed a height of 89 cm (50–75 p) and weight of 11 kg (10–25 p), and dry mucosa, tachypnea, and hyperpneic respiration, accompanied by confusion and hepatomegaly. The laboratory examination results were as follows: glucose 14 mg/dL (70–100 mg/dL); uric acid 12.41 mg/dL (2–8 mg/dL); alanine transaminase (ALT) 196 U/L (5–40 U/L); aspartate transaminase (AST) 150 U/L (5–42 U/L); ketone positivity in urine (+++); prominent metabolic acidosis in the blood gases examination—pH 7.10 (7.35–7.45), pCO2 11.5 mmHg (35–45 mmHg), HCO3 5.8 mM (21–28 mM), BE −24.6 mM (−5 to +3 mM), and anion gap 28.8 mM (10–14 mM); and lactate 93.6 mg/dL (4.5–20 mg/dL). A glucose infusion was started at a rate of 10 mg/kg/min, and bicarbonate support was provided. The glucose infusion was increased, and insulin therapy was added due to low blood glucose levels. Approximately 15 h later, the hepatomegaly was recovered. The ALT level was corrected in 36 h. In addition, the blood gases parameters were recovered; the blood glucose was regulated; and the lactate level was decreased to

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3.68 mg/dL. Results of blood samples obtained during hypoglycemia—adrenocorticotropic hormone (ACTH) 869 pg/mL (5–48 pg/mL); cortisol >50.0 mcg/dL (5–15 mcg/dL); growth hormone 14.9 ng/mL (>10 ng/mL); and insulin 0.08 mIU/L (2–13 mIU/mL)—ruled out possible endocrinologic diseases. A detailed medical history revealed that the patient consumed a considerable amount of fructose-containing foods (bananas and strawberries), and that she had experience long-term fasting because of a febrile illness. The clinical and laboratory data are presented in Table 1.

FBPase deficiency was suspected in the patient, who had hypoglycemia, metabolic acidosis, and increased transaminases, and the clinical and laboratory signs were recovered with appropriate fluid and bicarbonate therapy. According to genetic analysis, the patient was defined as homozygous for c.658delT mutation at exon 5 in the FBP1 gene.

Patient 2. A five-and-a-half-year-old male sibling of the patient suspected of having the fructose metabolism disorder was also hospitalized, due to the same complaints and signs. The boy's medical history revealed two previous hospitalizations, at the ages of two and one-half and 3 years, due to fainting, tremor, complaints of sweating, and marked hypoglycemia. The physical examination conducted at the age of three revealed a height of 102 cm (75–90 p), weight of 15 kg (50–75 p), and altered mental status; respiration was hyperpneic and tachypneic; and the liver was palpable 2 cm below the rib. The laboratory results were as follows: glucose 9 mg/dL (70–100 mg/dL); uric acid 7.3 mg/dL (2–8 mg/dL); AST 46 U/L (5–40 U/L); ALT 26 U/L (5–42 U/L). The blood gas analysis revealed marked metabolic acidosis—pH 7.05 (7.35–7.45), pCO2 25 mmHg (35–45 mmHg), HCO_3 8.4 mM (21–28 mM), BE −23 mM (−5 to +3 mM), and anion gap 31.9 mM (10–14 mM), with a presence of ketone in the urine (+++). The lactate level was 163.6 mg/dL (4.5–20 mg/dL). Results of blood samples obtained during hypoglycemia were ACTH 103.6 pg/mL (5–48 pg/mL), cortisol >50 mcg/dL (5–15 mcg/dL), insulin <2 IU/mL (2–13 mIU/mL), and growth hormone 3.32 ng/mL (>10 ng/mL). The patient was not definitively diagnosed, and a carbohydrate-rich diet was recommended. No new attack was observed during follow-up.

FBPase deficiency was also considered in this patient, as that diagnosis had been made for his sibling. According to genetic analysis, the patient was defined as homozygous for c.658delT mutation at the exon 5 in the FBP1 gene, so FBPase deficiency was definitively diagnosed. The parents were informed about the disease, and they were warned about the effects of long-term fasting and told that the patients should abstain from fructose rich-nutrients. The pedigree of the family is shown in Figure 1.

Table 1. Clinical and Laboratory Information of Patients in the Attack

Age (year), gender	2, female	3, male
Hospitalization history due to hypoglycemia	1 year ago	6 months ago
Vomiting	+	−
Syncope	−	+
Abdominal pain	−	−
Height (cm) and SDS	89 (0.51)	102 (1.76)
Weight (kg) and SDS	11 (−1.19)	15 (0.42)
BMI (%) and SDS	13.89 (−2.07)	13.79 (−1.57)
Hepatomegaly	+	+
Blood gas analysis		
pH (7.35–7.45)	7.10	7.05
PCO_2 (35–45 mmHg)	11.5	25
HCO_3 (21–28 mM)	5.8	8.4
BE (−5 to +3 mM)	−24.6	−23
Anion gap (10–14 mM)	28.8	31.9
Biochemical evaluation		
Glucose (70–100 mg/dL)	14	9
Urea(10–50 mg/dL)	43	31
Creatinine (0.2–1.2 mg/dL)	0.77	0.47
Uric acid (2–8 mg/dL)	12.41	7.3
ALT (5–40 U/L)	150	46
AST (5–42 U/L)	196	26
Albumin (3.5–5 g/dL)	5.3	4.7
Hormonal evaluation		
ACTH (5–48 pg/mL)	869	103.6
Cortisol (5–15 mcg/dL)	>50	>50
Insulin (<2–13 mIU/mL)	0.08	<2
Growth hormone (>10 ng/mL)	14.9	3.32
Metabolic evaluation		
Lactate (4.5–20 mg/dL)	93.6	163.6
Keton in urine	+++	+++
Tandem mass	−	Normal

ALT, alanine transaminase; AST, aspartate transaminase; SDS, standard deviation score; BMI, body mass index; ACTH, adrenocorticotropic hormone.

Genetic analysis

The two patients from family 1 were examined at Harran University Hospital; their clinical details are presented in Table 1. Informed consent to include the patients in this study was obtained from the parents. Blood samples from the two patients were available for genetic analysis, and DNA was extracted from whole blood using a salting out procedure. Primers were designed for PCR amplification of seven exons of the FBP1 gene (Table 2), and the amplification products were sequenced on an Applied Biosystems 3730xl automated sequencer. The FBP1 gene sequence analyses of patients 1 and 2 are shown in Figure 2. Based on the

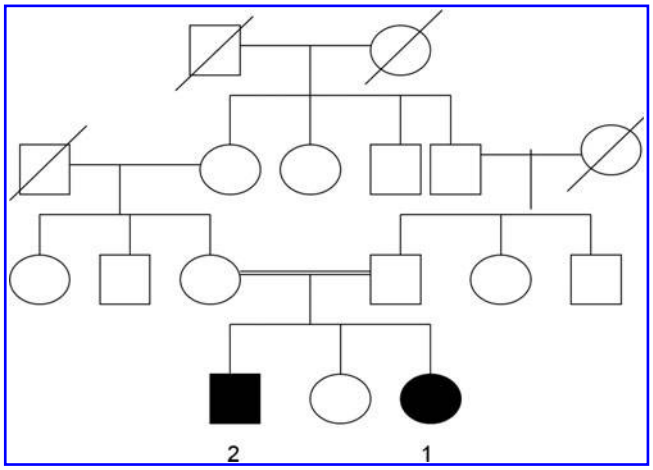

FIG. 1. Pedigree of probands; it is known that there is FBPase deficiency in patients 1 and 2.

Table 2. Genotyping of FBP1 Gene

Exon	*Forward*	*Reverse*	*Product size (bp)*
Promoter	5′-ACAGTGCCGGGTGGAGGGCAC-3′	5′CGCAGCGCTCGTGGTGCAAC-3′	274
1	5′-CAGTGCCTACTGCCCTCTCTT-3′	5′-AGGCTCCCCAGGCAGGCAGACAGACA-3′	313
2	5′-CTACATGTTCTGGTGGTCATGG-3′	5′-CTGGGAAGAAGACCGGCTACAT-3′	270
3	5′-CTCCTTTAGTGTATCTTGCT-3′	5′-CTTCTGTCCCCAAACCAAGTG-3′	193
4	5′-TTGAGAATGCCTCCTGTTAAT-3′	5′-TCATTTGCTCACAGACACCAG-3′	281
5	5′-ATCCAGGCCTGGGGACCCAG-3′	5′-CCCAGAACCTGCACCACCCTC-3′	238
6	5′-CACAGAAACTTAGGAGACACC-3′	5′-ATCTGCTCCTCACTCCCTCTC-3′	236
7	5′-TTGGAAACTCCCACCAGCTCT-3′	5′GAATGTAAGGTGCACAGCAGG-3′	385

Primers and PCR product for FBP1 gene exons and promoter regions (el-Maghrabi *et al.*, 1995).

sequencing results, FBP1 gene c658T deletion in exon 5 was determined. Because of this deleted T nucleotide, there was a frame shift mutation; all of the codon had changed, and in exon 6 TGA (stop codon), the codon had disappeared, and FBP1 protein could not be expressed effectively (Fig. 3).

Discussion

Fructose-1,6-bisphosphatase (EC 3.1.3.11) is known as the regulatory enzyme that hydrolyzes fructose-1,6-bisphosphate to inorganic phosphate and fructose 6-phosphate. FBPase deficiency, which is related to hypoglycemia and metabolic acidosis, is encountered less frequently than hereditary fructose intolerance; the incidence of FBPase deficiency was reported as 1:350,000 in the Netherlands (Visser *et al.*, 2004). While approximately half of subjects with FBPase deficiency show signs during the newborn period, they are symptomatic during long-term fasting due to febrile diseases in more advanced ages. Patients experience generally normal health between attacks; however, they may show signs after high-dose fructose intake in this disease. The attacks decrease with age, and the majority of cases exhibit normal somatic and psychomotor development (Baerlocher *et al.*, 1971). The disease was first defined in a 5-year-old child in 1970, and many patients were reported in the following years (Baker and Winegrad, 1970; Pagliara *et al.*, 1972). Kikawa *et al.* (1997) presented data of 13 children from 11 Japanese families, and reported that there was consanguinity in three families. In our cases, there was first-degree consanguinity between the parents. Contrary to hereditary fructose intolerance, gastrointestinal signs are few, and there is no restriction of fructose needed. In our two cases, there were no gastrointestinal signs, but they had severe acidosis and hypoglycemia. Both cases were life threatening, and the patients were treated under intensive care unit conditions. In cases of hypoglycemia and lactic acidosis, FBPase deficiency should be considered in the differential diagnosis, even though it is rarely encountered. In addition, fructose should be excluded from the diet and long-term fasting should be prevented.

The FBP1 gene, which is thought to be responsible for the disease, was defined on chromosome 9q22.2-q22.3 in 1995 by el-Maghrabi *et al.* (1995). The gene consists of seven exons and spans over 31 kb (el-Maghrabi *et al.*, 1995). The FBP1 gene has three transcripts (FBP1-201, FBP1-001 and FBP1-002

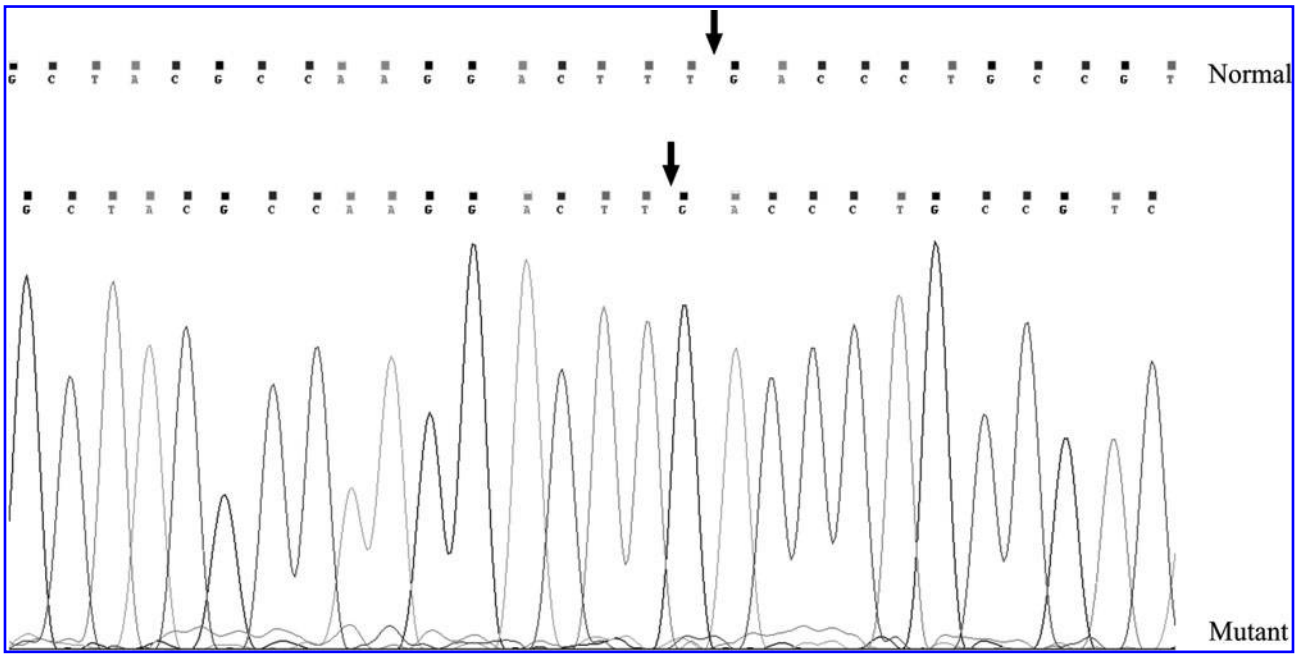

FIG. 2. Mutation analyses of patients′ FBP1 gene c.658 del T novel mutation. Arrows indicate deleted position.

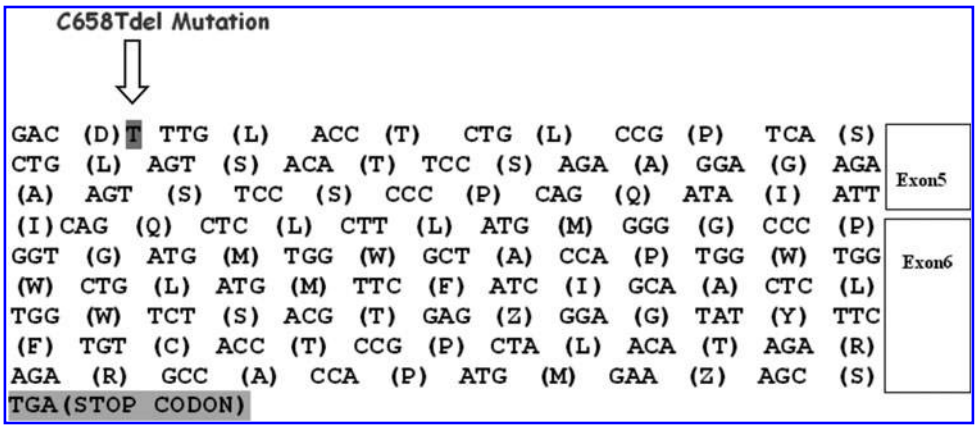

FIG. 3. The GAC (D) codon T nucleotide was deleted, resulting in frame shift mutation. The 1-bp T deletion (bp position 658) results in a shift of the reading frame and a stop codon after 56 amino acids.

splice variants), and the c.658delT mutation is not encoded across a splice junction. We think that FBP1 protein is not expressed clearly in these patients because of the c.658delT mutation, which results in FBPase deficiency. Homozygous and heterozygote mutations were determined in the study performed in Japan, and a 1 bp insertion (960insG) on the FBP1 gene was defined as the one most commonly encountered (10 out of 16 mutant alleles). In another study performed in Japan, two mutations, which were heterozygote on the FBP1 gene, were detected in a girl (Matsuura *et al.*, 2002). Eleven different FBP1 mutations were also defined in that study. Herzog *et al.* (1999) detected a homozygous mutation in exon 7 of 960-961insG on the FBP1 gene; in addition, mutations of 35delA in exon 1, 778GA and 966delC in exon 7 were detected for the first time. According to the study performed on Japanese patients, the c.961 ins G mutation was defined as 46% (Kikawa Y *et al.*, 1997). Other homozygous and heterozygous mutations belonging to the FBP1 gene were also defined in different studies performed on other patients with FBPase deficiency (Faiyaz-Ul-Haque *et al.*, 2009; Moon *et al.*, 2011). As such, different mutations were defined in FBPase deficiency diseases in different populations (Table 3).

Table 3. Mutations That Have Been Found in FBP1 Gene in Different Populations

Mutation	*Population*	*Reference*
1-bp insertion (960insG) in exon 7	Japan	Kikawa *et al.* (1995)
490G-A transition in exon 4	Japan	Kikawa *et al.* (1997)
530C-A transversion in exon 4	Japan	Kikawa *et al.* (1997)
88G-T transversion in exon 1	Japan	Kikawa *et al.* (1997)
581T-C transition in exon 5	Japan	Matsuura *et al.* (2002)
six nucleotide repetitive insertion, c114_119dup CTGCAC	Saudi Arabia	Faiyaz-Ul-Haque *et al.* (2009)
c.841G > T c.778G > A c.881G > A G164S or 838delT in exon 7	Sweden South Korea	Asberg *et al.* (2010) Moon *et al.* (2011)

Conclusion

Different from other studies, homozygous c.658delT mutation, which was not previously defined, was determined by FBP1 gene analysis of our patients with FBPase deficiency. Homozygous c.658delT mutation caused a stop codon in exon 6. Because of that, FBP1 gene expression is blocked at exon 6. Defective FBP1 protein is caused to FBPase deficiency in these patients. The present study is important because it is the first to report FBP1 gene novel mutation in our country.

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Disclosure Statement

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References

Asberg, C., Hjalmarson, O., Alm, J., Martinsson, T., Waldenström, J., and Hellerud, C. (2010). Fructose 1,6-bisphosphatase deficiency: enzyme and mutation analysis performed on calcitriol-stimulated monocytes with a note on long-term prognosis. J Inherit Metab [Epub ahead of print]; doi: 10.1007/s10545-009-9034-5.

Baerlocher, K., Gitzelmann, R., Nüssli, R., and Dumermuth, G. (1971). Infantile lactic acidosis due to hereditary fructose 1,6-diphosphatase deficiency. Helv Paediatr Acta **26,** 489–506.

Baker, L., and Winegrad, A.I. (1970). Fasting hypoglycemia and metabolic acidosis associated with deficiency of hepatic fructose-1,6-bisphosphatase activity. Lancet **2,** 13–16.

el-Maghrabi, M.R., Lange, A.J., Jiang, W., Yamagata, K., Stoffel, M., Takeda, J., Fernald, A.A., Le Beau, M.M., Bell, G.I., Baker, L., *et al.* (1995). 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 **27,** 520–525.

Emery, J.L., Howat, A.J., Variend, S., and Vawter, G.F. (1988). Investigation of inborn errors of metabolism in unexpected infant deaths. Lancet **2,** 29–31.

Faiyaz-Ul-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, S.H. (2009). Novel FBP1 gene mutations in Arab

patients with fructose-1,6-bisphosphatase deficiency. Eur J Pediatr **168,** 1467–1471.

Herzog, B., Wendel, U., Morris, A.A., and Eschrich, K. (1999). Novel mutations in patients with fructose-1,6-bisphosphatase deficiency. J Inherit Metab Dis **22,** 132–138.

Kikawa, Y., Inuzuka, M., Jin, B.Y., Kaji, S., Koga, J., Yamamoto, Y., Fujisawa, K., Hata, I., Nakai, A., Shigematsu, Y., Mizunuma, H., Taketo, A., Mayumi, M., and Sudo, M. (1997). Identification of genetic mutations in Japanese patients with fructose-1,6-bisphosphatase deficiency. Am J Hum Genet **61,** 852–861.

Kikawa, Y., Inuzuka, M., Jin, B.Y., Kaji, S., Yamamoto, Y., Shigematsu, Y., Nakai, A., Taketo, A., Ohura, T., Mikami, H., *et al.* (1995). Identification of a genetic mutation in a family with fructose-1,6- bisphosphatase deficiency. Biochem Biophys Res Commun **210,** 797–804.

Matsuura, T., Chinen, Y., Arashiro, R., Katsuren, K., Tamura, T., Hyakuna, N., and Ohta, T. (2002). Two newly identified genomic mutations in a Japanese female patient with fructose-1,6-bisphosphatase (FBPase) deficiency. Mol Genet Metab **76,** 207–210.

Moon, S., Kim, J.H., Han, J.H., Ko, S.H., Ahn, Y.B., Kim, J.H., Yang, S.H., and Song, K.H. (2011). Novel compound heterozygous mutations in the fructose-1,6-bisphosphatase gene cause hypoglycemia and lactic acidosis. Metabolism **60,** 107–113.

Pagliara, A.S., Karl, I.E., Keating, J.P., Brown, B.I., and Kipnis, D.M. (1972). Hepatic fructose-1,6-diphosphatase deficiency. A cause of lactic acidosis and hypoglycemia in infancy. J Clin Invest **51,** 2115–2123.

Steinmann, B., Gitzelmann, R., and Van der Berhge, G. (2001). Disorders of fructose metabolism. In: The metabolic and molecular bases of inherited disease. Scriver, C.R., Beaudet, A.L., Sly, W.S., and Valle, D, eds. (McGraw-Hill, New York), pp. 1489–1520.

Visser, G., Bakker, H.D., de Klerk, J.B.C., *et al.* (2004). Natural history and treatment of fructose 1,6-diphosphatase deficiency in the Netherlands. J Inherit Metab Dis **27 [Suppl 1],** 207(abstract).

Zammarchi, E., Donati, M.A., Ciani, F., Rubetti, P., and Pasquini, E. (1995). Fructose-1,6-diphosphatase deficiency misdiagnosed as Reye syndrome. Clin Pediatr (Phila) **34,** 561–564.

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