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Short communication

Segregation of a novel homozygous 6 nucleotide deletion in *GLUT2* gene in a Fanconi–Bickel syndrome family☆,☆☆

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ABSTRACT

Fanconi–Bickel syndrome (FBS) is a rare autosomal recessive disorder characterized by hepatorenal glycogen accumulation, proximal renal tubular dysfunction, impaired utilization of glucose and galactose, rickets, and severe short stature. It has been shown to be caused by mutations in *GLUT2* gene, a member of the facilitative glucose transporter family. Here, we report an Iranian family with 2 affected siblings. The clinical findings in the patients include developmental delay, failure to thrive, hepatomegaly, enlarged kidneys and rickets. A novel 6 nucleotide deletion (c.1061_1066del6, p.V355_S356del2) is shown to be segregated with the disease in this family.

1. Introduction

Fanconi–Bickel syndrome (FBS) or glycogen storage disease type XI is a rare genetic disorder, inherited in an autosomal recessive mode. The main features of this disorder are hepatorenal glycogen accumulation, proximal renal tubular dysfunction, impaired utilization of glucose and galactose, rickets, and severe short stature (Manz et al., 1987; Grunert et al., 2012). Characteristic laboratory findings of the disease are glucosuria, phosphaturia, generalized aminoaciduria, bicarbonate wasting, and hypophosphatemia due to proximal renal tubular dysfunction. Cases affected with this disorder have been reported from different locations including Europe, Turkey, Arab countries of the Near East and North Africa, Japan, and North America (Al-Haggar et al., 2011). It has been shown to cause by mutations in the *GLUT2* gene, a member of the facilitative glucose transporter family which is expressed in hepatocytes, pancreatic beta cells, and the basolateral membranes of intestinal and renal tubular epithelial cells (Mueckler et al., 1994). Here we report an Iranian family with 2 affected siblings with FBS.

2. Case reports

2.1. Patient 1

A ten-year-old girl, first child of healthy consanguineous Iranian parents (Fig. 1), was delivered spontaneously after an unremarkable pregnancy. Newborn screening results were unremarkable. The chief complaint of the patient was bone pain and difficulty walking. Failure to thrive and developmental delay were evident since she was 1 year old. Distended abdomen was apparent on physical exam. Abdominal ultrasound revealed hepatomegaly with bilateral kidney enlargement. Clinical and radiological signs of rickets were apparent. Laboratory assessment showed reduced serum calcium (7.9 mg/dL, normal: 8–10.2), reduced serum phosphorus (2.3 mg/dL, normal: 2.7–4.5), and markedly elevated serum alkaline phosphatase (2335 U/L, normal: 145–420) levels. Liver and kidney function tests were all normal. Lipid profile showed high total cholesterol (220 mg/dL) and triglyceride (335 mg/dL). Normal fasting blood sugar (84 mg/dL) and postprandial hyperglycemia (128 mg/dL) were recorded. Arterial blood gas analysis revealed metabolic acidosis (pH, 7.18; bicarbonate, 15 mmol/L) with normal anion gap. Serum sodium (Na^+) was 137 mmol/L, and serum potassium (K^+) was 3.1 mmol/L. Urine analysis showed glucosuria, proteinuria, and uric aciduria. Liver biopsy showed marked glycogen accumulation in hepatocytes. Parents were assessed for possible biochemical disorder. The results particularly for glucosuria were unremarkable.

Abbreviations: FBS, Fanconi–Bickel syndrome; GLUT, glucose transporter; PCR, polymerase chain reaction; Ser, serin; Val, valin.

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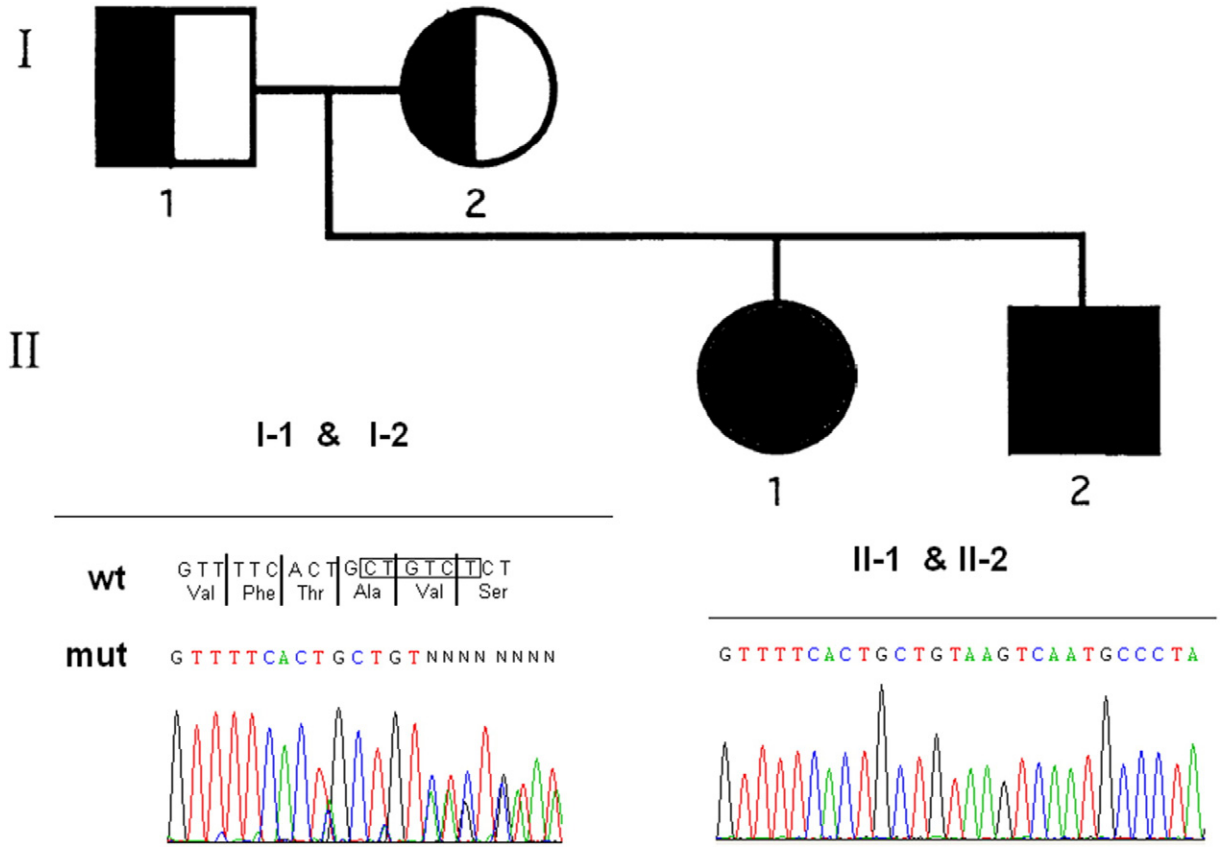

Fig. 1. The family pedigree and sequence chromatograms of patients and their parents. Deleted nucleotides are shown in rectangles.

2.2. Patient 2

Pregnancy and birth of the 8-month-old brother of patient 1 were also unremarkable as were newborn screening results. Abdominal ultrasound revealed hepatomegaly with both kidneys enlarged. Laboratory assessment showed signs of metabolic acidosis and hypophosphatemic rickets.

3. Material and methods

Informed consent was obtained from parents prior to participation in the study according to the protocol approved by the local institutional review board. Blood samples were collected from two siblings and their parents. Genomic DNA was isolated using the standard salting out method. PCR for *GLUT2* gene exons was performed according to previous studies with the same primers and PCR conditions (Santer et al., 2002). Molecular genetic analysis of PCR products, each containing one of the *GLUT2* exons and adjacent intronic segments, was performed based on the Sanger method with an automated ABI sequencer. The mutated cDNA sequence was submitted to the translate tool of the Expasy website (http://web.expasy.org/translate/) in order to detect the effect of the mutation on the protein sequence. Both native and mutated protein sequences were modeled with the use of the online server of Modeller homology modeling program (ModWeb) (Eswar et al., 2003). The obtained structures were further refined using the homology model refinement macro of YASARA (v.13.9.8) (Krieger et al., 2004). Representation of the protein was done with VMD 1.8.7 (Humphrey et al., 1996).

4. Results

A 6 nucleotide deletion was detected in exon 7 of both patients in homozygous state in both parents in heterozygous state (c.1061_1066del6, p.V355_S356del2). These 2 aminoacids are highly conserved during evolution. This deletion causes no frame shift. Translation of the nucleotide deletion showed that upon expression, the protein would be affected by deletion of Val355 and Ser356. Since there is no reported structure for the GLUT2 protein, homology models of the native and mutated

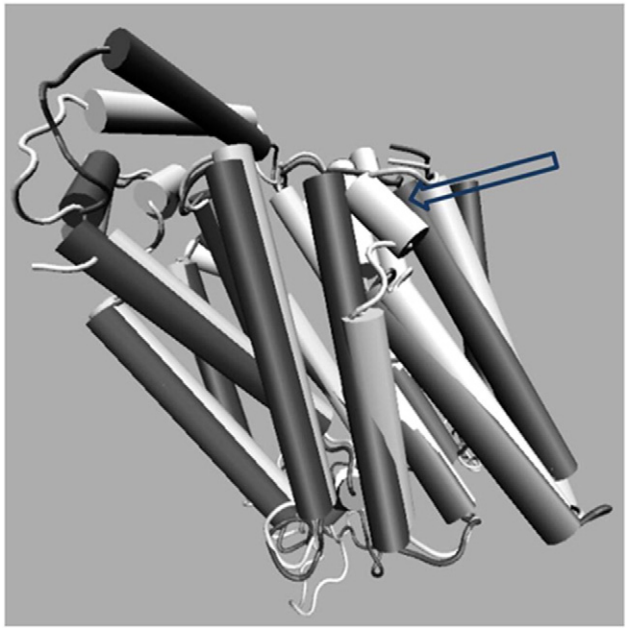

Fig. 2. Superimposed model structures of the native (dark gray) and mutated (light gray) GLUT2. The arrow indicates the helix segment that appears in the mutated protein.

Table 1
Summary of Fanconi–Bickel cases reported from Iran.

Patient no.	Sex	Novel clinical presentations	Mutation	The state of mutation	Reference
1	Female	Pseudotumor cerebri and liver failure	IVS8+1G>T	Homozygous	Karamizadeh et al. (2012)
2	Female	Neonatal diabetic ketoacidosis	C.963+1G>A	Homozygous	Setoodeh and Rabbani (2012)
3	Female	Frequent bone fractures	C.685_70ldel GCCATCCTTCAGTCTCT ins CAGAAAA	Homozygous	Hadipour et al. (2013)

proteins were generated with the use of ModWeb. The models are based on 4PYP.pdb, corresponding to the GLUT1 glucose transporter and comprise residues 7 to 485. The superimposed structures of proteins are shown in Fig. 2.

5. Discussion

The two patients reported here harbor all previously reported characteristics of FBS and were clinically diagnosed as FBS. None of the parents had glucosuria, an observation that is in concordance with Grünert et al. who showed that a deletion and a point mutation in the parents of reported patients have no clinical consequence (Grunert et al., 2012). However, Sakamoto et al. had suggested that missense mutations have a dominant negative effect on the facilitative glucose transporters that act as polymers (Sakamoto et al., 2000). Up to now, different mutations, mostly homozygous, have been found in different ethnic populations. It has been concluded that the prevalence of *GLUT2* mutation is relatively low in most populations (Santer et al., 2002). In addition to our study, 3 novel mutations have been found in Iranian patients diagnosed with FBS (Table 1) with all of them being homozygote mutations (Karamizadeh et al., 2012; Setoodeh and Rabbani, 2012; Hadipour et al., 2013). Consequently, genetic counseling is of significant importance for consanguineous couples, in order to ensure that they are correctly informed about the possible consequences before having a pregnancy. The mutation found in our patients was seen in homozygote state which is in concordance with the low prevalence of this disorder in the Iranian population. Since most mutations identified so far are novel mutations, molecular analysis should be done in greater number of families to provide evidence for the most frequent mutations in order to establish screening molecular tests (Al-Haggar et al., 2011).

The closest reported protein structure to GLUT2 is the related GLUT1, a member of the same sugar porter subfamily, whose crystal structure has been reported very recently (Deng et al., 2014). Residues Val 355 and Ser 356 of GLUT2 correspond to Val 323 and Ser 324 of GLUT1, of which Ser 324 has been observed to be mutated to Leu in absence epilepsy (Mullen et al., 2010). These residues are located in the "C domain" (C-terminal domain) which is suggested to be involved in substrate binding (Deng et al., 2014). The Val 355 and Ser 356 deletion would result in a change of structure in GLUT2: as shown in Fig. 2. The (predicted) TM8 helix in the normal protein would split into two helices. This change may also affect the overall conformation of the protein, as some other segments of the two proteins may also not superimpose completely. In brief, in silico analyses suggest that this deletion could affect the function of corresponding protein.

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