

BRIEF REPORT —MUTATION REPORT

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Mutation analysis of two Japanese patients with Fanconi-Bickel syndrome

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Abstract Fanconi-Bickel syndrome (FBS), or glycogen storage disease type XI, is a rare autosomal recessive disorder characterized by hepatorenal glycogen accumulation, Fanconi nephropathy, and impaired utilization of glucose and galactose. Recently, this disease was elucidated to link mutations in the glucose transporter 2 (*GLUT2*) gene. Only three mutations in three FBS families have been reported. Therefore, it is important to elucidate mutations in the *GLUT2* gene in FBS by answering the question of whether the syndrome is a single gene disease. In this report, we describe two patients in two unrelated families clinically diagnosed with FBS. No mutation in the entire protein coding region of the *GLUT2* gene was detected in patient 1, which suggested that no mutation existed in the *GLUT 2* gene, or that some mutations had affected the expression of the *GLUT 2* gene. In patient 2, a novel homozygous nonsense mutation (W420X, Trp at codon 420 to stop codon) was detected. These results support the correlation between *GLUT2* gene mutation and FBS syndrome. However, many patients must be analyzed to determine whether other genes are involved in FBS.

Key words Fanconi-Bickel syndrome · Glycogen storage disease type XI · Glucose transporter 2 · Nonsense mutation · Japanese patient

Introduction

Fanconi-Bickel syndrome (FBS), or glycogen storage disease type XI, is a rare autosomal recessive metabolic disorder characterized by hepatorenal glycogen accumulation, Fanconi nephropathy, and impaired metabolism of glucose

and galactose. The disease was first described by Fanconi and Bickel in 1949 (Fanconi and Bickel 1949) and is also known by other names, such as glycogen storage disease type XI. However, the underlying enzymatic defects in carbohydrate metabolism have not been identified. In 1997, Santer et al. (Santer et al. 1997) reported mutations in the glucose transporter 2 (*GLUT2*) gene in this disease.

The structural gene encoding *GLUT2* has been cloned and mapped to chromosome 3q26.1–26.3 (Takeda et al. 1993). The *GLUT2* gene contains 11 exons spanning 30kb. To date, three mutations in the *GLUT2* gene have been reported in FBS (Santer et al. 1997). In their recent review of FBS, Santer et al. (1997) described 82 cases from 70 families in the published literature and their personal communications, but only 14 were confirmed, including the reported cases of FBS determined by detection of mutations within the *GLUT2* gene. Therefore it is important to elucidate molecular defects to determine whether or not the syndrome is a single gene disease. In this report, we describe two patients with FBS; one had a novel nonsense mutation, but no mutation was detected in the other. This is the first report of the *GLUT2* gene mutation in a Japanese patient with FBS.

Case reports

Patient 1

The patient was a 23-year-old Japanese woman born by normal delivery of non-consanguineous parents. At 5 months of age, her mother noticed marked distention of her abdomen. Hepatomegaly, glucosuria, and short stature were noted by a local doctor. At 10 months of age, she was diagnosed with renal glucosuria and rickets caused by renal tubular dysfunction. At 4 years of age, she was referred to the National Children's Medical Research Center for a liver biopsy. She was diagnosed with glycogen storage disease with Fanconi syndrome because of increased glycogen accumulation, with normal enzymatic activities of hepatic glu-

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cose-6-phosphatase, glycogen-debranching enzyme, and phosphorylase kinase. She also exhibited galactosuria. Treatments were supplementation of active vitamin D, phosphorus, and bicarbonate, and taking uncooked cornstarch before sleep. Her bone abnormalities, especially genu valgum, gradually progressed. She received an operation for bone deformities. Her final height was 135.0cm (-4.3 SD) and body weight was 36.4kg (-2.3 SD). Her two sisters were healthy.

Patient 2

The patient was a 21-year-old Japanese man born by normal delivery from consanguineous parents (his grandmothers were cousins). Poor weight gain was detected at 1 month of age, and hepatomegaly and glucosuria were found. He was diagnosed with Fanconi syndrome at 3 months of age. At the age of 1 year and 6 months, he received a liver biopsy for definitive diagnosis, but enzymatic activities of glycogen metabolism were all normal, despite an increased amount of glycogen. He also showed galactosuria and was diagnosed with glycogen storage disease and Fanconi syndrome, and was treated with vitamin D, phosphorus, and bicarbonate. He was also given uncooked cornstarch before sleep. At the age of 20 years, brain computed tomography scans showed calcification of the bilateral basal ganglia, while renal calcification was detected by ultrasonography. His final height was 148.5cm (-3.0 SD) and body weight was 37.0kg (-2.8 SD). His sister was healthy.

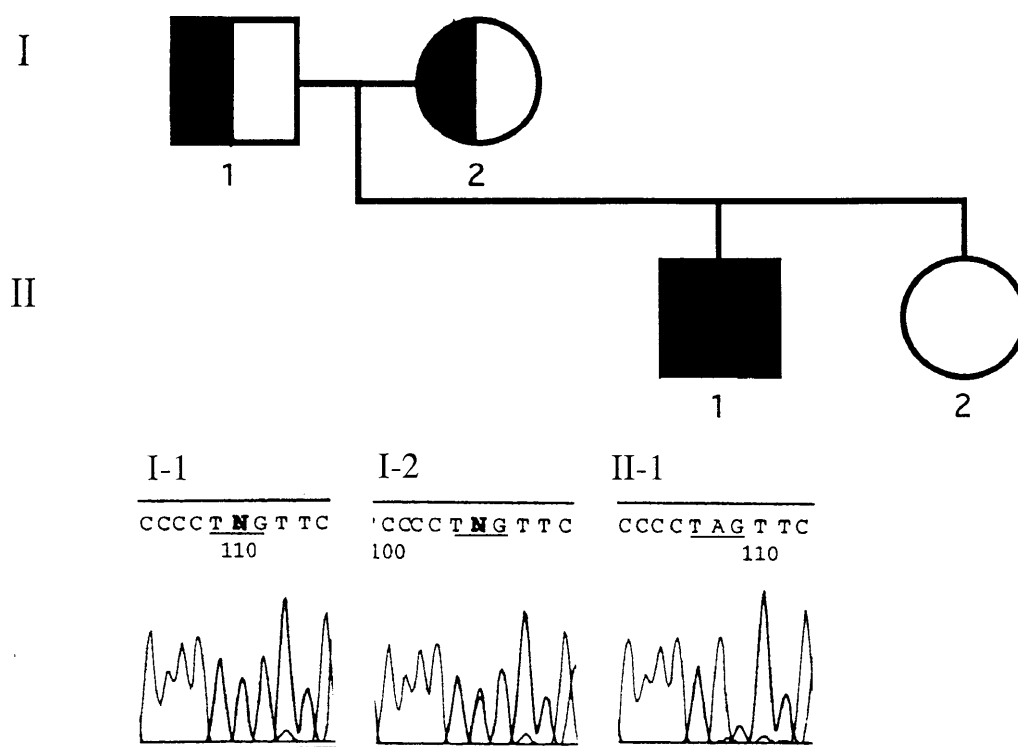
Materials and methods

Genomic DNA was isolated from peripheral blood leukocytes of the patients and their parents by standard methods. All 11 exons of the *GLUT2* gene, including the exon/intron boundary, were amplified from genomic DNA by polymerase chain reaction (PCR) methods, using the primer sets noted by Takeda et al. (1993). In patient 1, total RNA was isolated from cultured skin fibroblasts and subjected to reverse transcribed-PCR (RT-PCR) amplification. The amplified products were purified and directly sequenced with a dye terminator cycle sequencing ready reaction kit (ABI 310 Genetic Analyzer, Perkin-Elmer, Foster City, CA, USA).

Results and discussion

FBS is characterized by hepatorenal glycogen storage, hypoglycemia, hypergalactosemia, and proximal renal tubular dysfunction. Recently *GLUT2* gene mutations were reported in FBS (Santer et al. 1997). The two patients reported here had all the characteristics of and were clinically diagnosed with FBS. However, DNA sequence analysis revealed that patient 2 had a G-to-A transition at cDNA nucleotide number 1159 in exon 9 of the *GLUT2* gene, resulting in a Trp to stop codon at codon 420 (TGG to TAG, designated as W420X) (Fig. 1). No mutation was detected in the entire protein coding region of the *GLUT2*

Fig. 1. Direct sequence analysis of genomic DNA of the *GLUT2* gene. Direct sequence analysis of the exon 9 region in patient 2 showed a homozygous amino acid of codon 420 (TAG; underlined) in the patient, and a heterozygous amino acid of the codon (TGG and TAG; underlined) in his parents. The peak heights of G and A were the same and the analyses were designated N



gene in patient 1. The mutation in patient 2 was confirmed to be heterozygous (G/A) in his parents for the W420X mutation (Fig. 1).

To examine mRNA in patient 1 and a control, RT-PCR was performed using cultured skin fibroblasts from the patient and control. However, it was impossible to amplify cDNA of the *GLUT 2* gene from normal cultured skin fibroblasts, probably because there was no expression of the gene in the cells (Fukumoto et al. 1988). In patient 1, two possibilities were considered; no mutation existed in the *GLUT 2* gene, or, alternatively, some mutations affected the expression of the *GLUT 2* gene. Moreover, there was a low possibility that deletion mutations were present in the primer sequence.

To date, four mutations in the *GLUT2* gene, including the one in this report, have been reported in FBS; three were nonsense mutations and one was a deletion (Santer et al. 1997). All reported mutations had a stop codon, resulting in truncated protein without functional monosaccharide transport activity. However, there are reports of a missense mutation in the *GLUT2* gene in a diabetic patient and amino acid polymorphisms in the normal population (Mueckler et al. 1994; Matsubara et al. 1995; Shimada et al. 1995). In FBS, the reported mutations are very few, and no missense mutation has been reported. The phenotype of a missense mutation of the *GLUT 2* gene is speculative as is the correlation of diabetes and *GLUT2* gene mutations. Moreover, from the limited evidence it cannot be concluded that FBS is a single gene disease caused by *GLUT2* gene

mutations. Further mutation analysis could disclose whether FBS is a single gene disease or whether it is a syndrome derived from different gene loci.

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