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Mutation analysis of *SLC37A4* in a patient with glycogen storage disease-type Ib

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Abstract

Objective: The aim of the study was to investigate the relationship between *SLC37A4* gene mutation and clinical phenotype in a patient with glycogen storage disease-type I.

Methods: The clinical data of one patient with glycogen storage disease-type I accumulation syndrome and the results of *SLC37A4* gene testing were analyzed. DNA from peripheral blood was used to analyze the *SLC37A4* mutations of the patient and his parents.

Results: The patient carried a compound heterozygous mutation of *SLC37A4*, his mother was heterozygous for the c.572C > T (p.P191L) mutation, and his father was heterozygous for the c.359C > T (p.P120L) mutation.

Conclusion: The patient had two gene mutations: c.359C > T (p.P120L), which is closely related to glycogen storage disease-type I, and c.572C > T (p.P191L), which is a known mutation in the disease.

Keywords

Glycogen storage disease-type I, SLC37A4 gene, glucose 6 phosphate transferase, gene mutation, compound heterozygote, growth hormone

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Introduction

Glycogen storage disease type I (GSD-I) is characterized by lactic acidosis, fasting hypoglycemia, kidney enlargement, growth retardation, hyperuricemia, hyperlipidemia, and hepatomegaly. Clinical manifestations of GSD-I include craniofacial anomalies, myelomeningocele, short stature, sacral

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agenesis, intellectual disability, speech delay, pectus excavatum, and abnormal behavior.[1] The incidence rate of GSD-I is approximately 1/100,000,[2] of which GSD-Ib accounts for 20%.[3] GSD-I includes two major subtypes: GSD type Ia (GSD-Ia, MIM #232200), caused by a deficiency in glucose-6-phosphatase (G6Pase, EC 3.1.3.9), and GSD type Ib (GSD-Ib, MIM #232220), resulting from a deficiency in glucose-6-phosphate translocase (G6PT1).[4] G6Pase consists of glucose-6-phosphate catalytic subunit (G6PC) and glucose-6-phosphate transporter (G6PT), and it plays an important role in glycogen synthesis and decomposition.[4] GSD-Ib is an autosomal recessive disease caused by a mutation in the *SLC37A4* gene.[5–7] Compared with patients with GSD-Ia, those with GSD-Ib are more susceptible to bacterial infections due to neutropenia and neutrophil dysfunction.[2,3] Analysis of the *SLC37A4* gene is particularly important for the diagnosis and prognosis of GSD-Ib.[8]

GSD-Ib is also an immune disorder, which presents with oral and intestinal mucosal ulcers, enteritis, immune thyroiditis, growth hormone deficiency, and immune myasthenia gravis.[9–11] Since the *SLC37A4* gene was first identified, 96 disease-causing mutations have been reported.[12] Mutations of *SLC37A4* in Chinese patients reported so far have come from the mainland (23 cases), Hong Kong (3 cases), and Taiwan (1 case).[13] In this study, we performed a mutation analysis of *SLC37A4* in a patient with GSD-Ib to further elucidate the correlation between *SLC37A4* mutation and clinical phenotype.

Subject and methods

Ethical approval was obtained from Beijing Jishuitan Hospital Ethics committee. Written informed consent obtained from the patient's parents.

Clinical description

A 9-year-old boy was admitted to our hospital because of slow growth for 3 years. He was a full-term infant of a gravida 2 para 1 mother. At 3 years of age, he was admitted to Beijing Children's Hospital because of enlargement of the liver and spleen and anemia that had lasted for 1 month. He was diagnosed with GSD-Ib according to clinical and laboratory test results, including short stature, hepatosplenomegaly, history of recurrent infection, hypoglycemia, hyperlipidemia, hyperuricemia, hyperlactacidemia, and low insulin. After discharge, the patient was given raw corn starch 2 g/kg once every 4 to 6 hours. After oral administration of corn starch, the patient's blood glucose remained stable and no significant hypoglycemia occurred. On physical examination at 9 years of age, the patient weighed 25 kg, was 120 cm tall, and had a baby face. A cardiopulmonary examination showed no abnormality and the liver could be felt 5 cm below the ribs. His parents were asymptomatic, married, and nonconsanguineous. Laboratory test results were as follows: white blood cell count: 3.27×10^9/L, absolute neutrophil count: 0.57×10^9/L, hemoglobin: 118 g/L, blood platelet: 281×10^9/L, alanine aminotransferase: 5 U/L, aspartate transaminase: 48 U/L, alkaline phosphatase: 136 U/L, glutamine aminotransferase: 14 U/L, triglyceride: 1.89 mmol/L, cholestenone: 3.23 mmol/L, fasting blood glucose: 3.1 mmol/L, and blood uric acid: 595 mol/L. The patient had normal thyroid function and electrocardiogram, and abdominal ultrasound showed a slightly enlarged liver and enlarged spleen.

Mutation analysis

A sample (2 mL) of peripheral blood was collected from the proband and his parents. Genomic DNA was isolated from peripheral blood leukocytes by using the Blood DNA

Extraction Kit (Tiangen, Beijing, China). Then, genomic libraries were constructed, and the exons and adjacent intron regions (50 bp) of the gene associated with GSD were captured by probe hybridization and enriched. The enriched targeted gene fragments were sequenced by next-generation sequencing (Illumina Inc., San Diego, CA, USA). The sequencing data were compared with the human hg19 reference genome provided by the University of California Santa Cruz (UCSC) database using Next Gene V2.3.4 software (Virgilbio, Beijing, China), and the coverage of the target area and the quality of the sequencing were evaluated. All mutations that could potentially cause the disease were subjected to Sanger sequencing (ABI 3130 sequencer, Applied Biosystems/Thermo Fisher Scientific, Waltham, MA, USA) for verification. Pathogenicity prediction analysis was carried out on the SIFT (sorts intolerant from tolerant amino acid substitutions, http://provean.jcvi.org/protein_batch_submit.php?species=human) and Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) platforms.

Growth hormone therapy

A hormone stimulation test showed that the level of growth hormone (GH) was normal; the peak value of GH was 11.8 ng/L at 90 minutes. Because the peak value was greater than 10 ng/L and the value of insulin-like growth factor (IGF-1) (67.8 ng/L) was lower than normal (74–388 ng/L), we performed the IGF-1 generation test. The patient was treated with 7.5 IU of GH once a day for 4 days, after which IGF-1 was 169 ng/L, indicating that the IGF-1 generation test was effective. Because IGF-1 levels are mainly regulated by GH and are an important indicator for the diagnosis of GH deficiency, the test of IGF-1 generation was performed. The patient was treated with 7.5 IU/d GH once a day for 4 days. After that, IGF-1 was improved to normal level 169 ng/L, so IGF-1 generation test was effective. According to the growth pattern and delayed bone age, we initiated treatment with recombinant human GH (GenSci, Changchun, China) at a dosage of 4 IU/day, as shown in Table 1. At the age of 11 years to 11 years 4 months, 11 years 4 months to 11 years 7 months, and 11 years 7 months to 12 years 10 months, the GH dosage was adjusted to 4.5, 5, and 5.5 IU/d, respectively. The patient was followed up once a month; weight, height, and growth rate were recorded; and secondary sexual characteristics and local injection reactions were observed. Bone age of the patient was examined every 3 months. Considering the possible adverse effects of GH therapy, including arth4ralgia, hypothyroidism, headaches, and hypertension, we monitored the patient's biochemical parameters, including IGF-1, blood routine leukocyte, neutrophil absolute value, triglycerides, and uric acid (Table 1).

Results

Sequence analysis

The patient carried a compound heterozygous variation of *SLC37A4*: his mother was a heterozygous carrier of c.572C > T (p.P191L) (Figure 1), and his father carried c.359C > T (p.P120L) (Figure 2) of the *SLC37A4* gene. The protein function prediction platforms SIFT and PolyPhen_2 showed that the two missense variants of *SLC37A4* in this study were pathogenic. These results confirmed the diagnosis of GSD-Ib in this patient.

GH therapy

After initial treatment with GH, the patient's growth improved significantly. The patient showed no clinical manifestations of acidosis or hypoglycemia before or during GH

Table 1. Patient data on metabolic status during growth hormone treatment.

Months of treatment	Age (years–months)	Bone age (years)	Height (cm)	Weight (kg)	IGF-1 (ng/mL)	Leucocytes (10^9/L)	Absolute neutrophils (10^9/L)	Triglyceride (mmol/L)	Uric acid (mol/L)	FBG (mmol/L)	Glycosylated hemoglobin (g/L)
Baseline	9–10	8	120	25	67.8	3.27	0.57	1.89	595	3.1	4.6
2	10	–	121.4	25	121	4.27	2.38	2.09	624	3.7	5
6	10–4	–	124.6	27	174	4.45	0.92	2.21	557	3.6	5
9	10–7	–	127.4	28.7	232	–	–	2.79	612	3.5	4.6
12	10–10	9	129.7	29.4	128	3.78	1.02	2.45	685	3.8	4.7
14	11	–	130.6	29.85	94.8	–	–	2.12	647	3.5	4.8
18	11–4	–	132.4	31	114	–	–	2.02	604	3.6	5
21	11–7	–	134	32.1	129	–	–	3.12	729	3	4.8
24	11–10	10	135.2	32.55	187	3.53	0.75	2.27	694	3.5	4.7
30	12–4	–	135.2	34	72.7	2.86	0.57	2.4	586	3	4.5
36	12–10	11	137.7	32	88	2.6	0.31	4.44	751	3	4.1

IGF-1, insulin-like-growth factor 1; FBG, fasting blood sugar.

Figure 1. Heterozygous mutation c.572C > T (p.T191L) in the *SLC37A4* gene of the patient (maternal source).

Figure 2. Heterozygous mutation c.359C > T (p.P120L) in the *SLC37A4* gene of the patient (paternal source).

therapy. Biochemical parameters showed no increase in liver transaminase, although a slight increase in uric acid level occurred in the course of treatment. The patient's initial height was 120 cm, increasing to 129 cm after 12 months of treatment with GH. The patient grew 5.5 cm between the age of 10 years 10 months and 11 years 10 months. The patient's height reached 135.2 cm after 3 years of treatment with GH. His growth velocity increased remarkably, as shown in Figure 3. Based on the growth curves of height and weight in Chinese boys from 2 to 18 years (Figure 3),

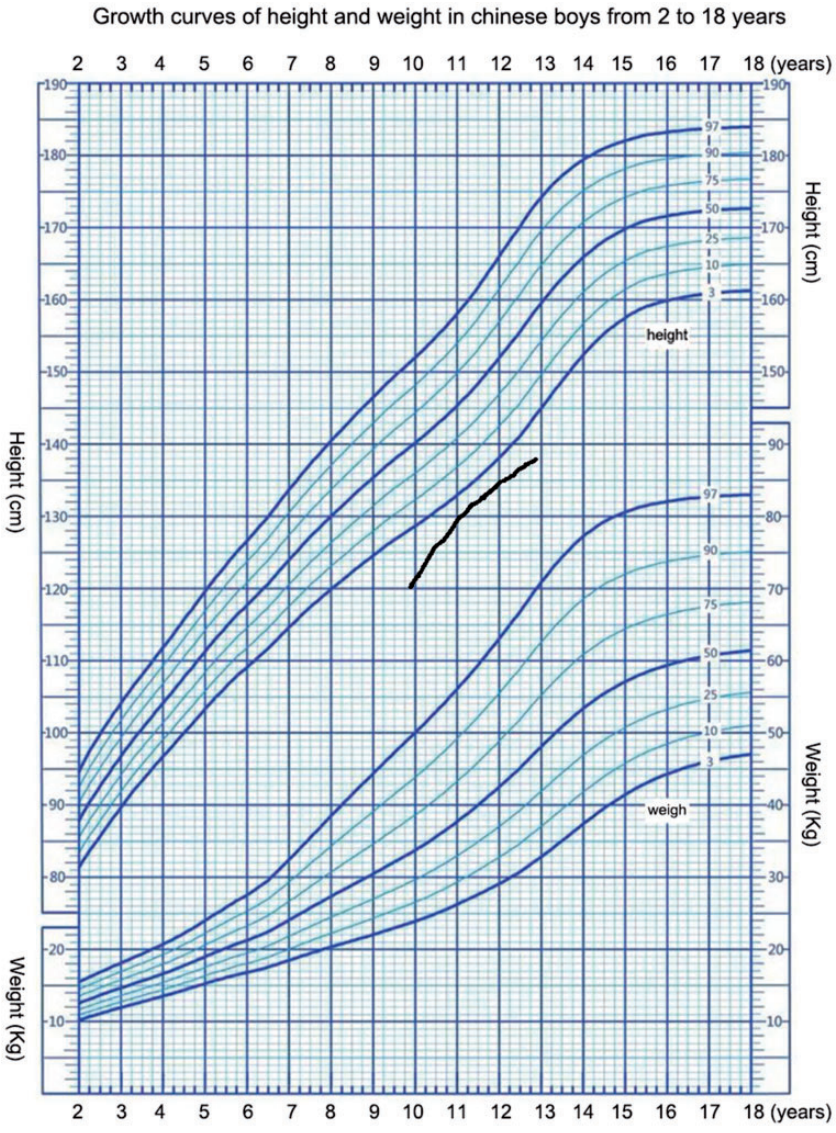

Figure 3. Growth curves of height and weight in Chinese boys from 2 to 18 years of age. The growth curve of the proband after growth hormone therapy was indicated by the black curve.

Table 2. Therapeutic effect of growth hormone treatment on the patient's height.

Month of treatment	Age (years)	Height (cm)
0	3 years 9 months	87
9	4 years 6 months	93
20	5 years 5 months	100.5
37	6 years 10 months	111
49	7 years 10 months	114
61	8 years 10 months	116

the patient had a good response to GH treatment compared with raw corn starch treatment (Table 2). At the age of 11 years and 10 months, the patient stopped taking GH.

Discussion

Patients with GSD-Ib are mainly treated through dietary modification and oral corn starch to maintain blood sugar stability and growth without significant developmental delay.[14] Previous studies have demonstrated that the symptoms of GSD Ib (OMIM: 232220) and GSDIc (OMIM: 232240) caused by variations in the *SLC37A4* gene include hepatomegaly, hypoglycemia, and podagra (gout).[2,15–17] To elucidate the cause of the disease, nucleotide sequence analysis of a patient's blood must be carried out. To date, 16 *SLC37A4* gene mutations have been reported in 27 Chinese patients, including 8 missense mutations.[12,13,18] Of these, the most common mutations are p.Gly149Glu (p.G149E; 13/45) and p.Pro191Leu (p.P191L; 12/45).[7,19] In the current study, we found that the patient's mother carried the c.572C > T (p.P191L) mutation. Lam et al.[7] analyzed the *G6PT1* gene of a Chinese family with GSD1b and found yjtrr family members to be heterozygous carriers of c.572C > T (p.P191L) (maternal source), and the other locus was c.446G > A (p.G149E) (paternal source). In another study, a patient was heterozygous for the c.572C>T (p.P191L) (paternal) mutation of *SLC37A4* and for c.70T > C (p.Y24H) (maternal).[20] In addition, Chen et al.[21,22] demonstrated that the mutation inhibited the activity of microsomal glucose 6-phosphorylase by functional test. However, there have been no previous reports regarding the heterozygous c.359C>T (p.P120L) mutation of paternal source, as found in the current study, and this mutation has not been detected in a database of healthy individuals. In this study, GH treatment was effective and safe, which is consistent with previous reports.[23,24]

In the current study, we performed a mutation analysis of the *SLC37A4* gene and summarized the clinical features of a patient with GSD-Ib. We found a c.359C > T (p.P120L) mutation in *SLC37A4*, which we confirmed as the cause of the disease in this patient. For patients with clinically suspected glycogen accumulation, further examination of pre- and postprandial adrenaline is required. If the blood glucose increase is less than 2.5 mmol/L, the patient is confirmed to have GSD-I. If the patient has a history of peripheral blood neutropenia, repeated oral ulcers, or joint pain, the patient is more likely to have GSD-Ib. Therefore, patients are advised to undergo *SLC37A4* genetic testing to clarify the diagnosis of GSD-Ia or GSD-Ib.

Author contributions

YZ drafted the manuscript. HS and NW were the lead clinicians for this case; they reviewed the treatment options and formulated and carried out the management plan. All authors read and approved the final manuscript.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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