

Molecular genetic analysis and phenotypic characteristics of a consanguineous family with glycogen storage disease type Ia

YILI LU^{1*}, LIYIN WANG^{2*}, JUN LI³, BEIBEI WU¹, HUIPING WU¹, YUE LUO¹, ZI-BING JIN⁴ and XIAOOU SHAN¹

¹Department of Pediatrics, The Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang 325027;

²College of Basic Medical Sciences, Capital Medical University, Beijing 100069; ³Department of Hand Surgery, Wenzhou Donghua Hospital; ⁴Division of Ophthalmic Genetics, Laboratory for Stem Cell and Retinal Regeneration, The Eye Hospital of Wenzhou Medical University, Wenzhou, Zhejiang 325027, P.R. China

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Abstract. Glycogen storage disease type-Ia (GSD-Ia) is a rare autosomal recessive disease caused by a mutation in the gene encoding glucose-6-phosphate- α (G6PC). The present study reported the case of a 3-month-old female Chinese patient with GSD-Ia born to consanguineous parents. The aim of the present study was to identify the precise mutation of the *G6PC* gene associated with this family and to describe the phenotypic characteristics of the patient. A comprehensive examination was performed on the patient, including physical examination, vein blood gas analysis, abdominal sonography and biochemical analyses. In addition, gene sequencing was performed on the coding region of the *G6PC* gene to identify the mutation. The patient was diagnosed with GSD-Ia and a *G6PC* missense mutation of c.518T>C (p.L173P) located in a highly conserved area was identified. The mutation is in a non-helical region of the protein, which previous studies have suggested should result in a lesser effect on G6PC enzymatic activity and milder phenotypic characteristics compared with mutations located in helical regions. However, the severity of the disease phenotype in the subject of the present study was inconsistent with that predicted from her genotype. The patient suffered from serious hypoglycemia, lactic acidosis, increased

triglycerides, hepatic dysfunction, clear hepatomegaly and nephromegaly. The incidence of the p.L173P mutation may be relatively high in the Chinese population. Knowledge of the various phenotypic presentations of the p.L173P mutation may be beneficial for future investigations.

Introduction

Glycogen storage disease-type I (GSD-I), first described by von Gierke in 1929 (1), is an inherited deficiency of the glucose-6-phosphatase (G6Pase) complex. G6Pase is composed primarily of glucose-6-phosphatase- α (G6PC) and glucose-6-phosphate translocase (G6PT) (2). Abnormalities in G6PC and G6PT result in GSD-Ia and GSD-Ib, respectively, which are the primary subtypes of GSD-I (2).

G6PC is a glycoprotein anchored in the endoplasmic reticulum membrane by nine transmembrane helices. G6PC is vital for the maintenance of glucose homeostasis as it catalyzes the final step in the glycogenolytic and glyconeogenic pathways (2,3), and is therefore present in numerous glucose-generating organs, including the liver, kidney and small intestine (4,5). G6PC deficiency or abnormality may result in GSD-Ia (2,6). GSD-Ia is characterized by hypoglycemia, growth retardation, lactic acidosis, high levels of lipids, hepatomegaly and nephromegaly. Due to these clinical manifestations, patients with GSD-Ia are typically diagnosed in childhood, and the diagnosis may be confirmed using gene sequencing to confirm the presence of mutations in *G6PC* (6,7). G6PT, the protein involved in GSD-Ib, is a transporter that functions to facilitate glycogen entry into the cell. Similar to GSD-Ia, GSD-Ib is characterized by hypoglycemia, high levels of lipids and hepatomegaly. However, patients with GSD-Ib may additionally suffer from impaired neutrophil function and neutropenia, resulting in recurrent bacterial infections (2).

GSD-Ia is responsible for >80% of GSD-I cases (4). The overall incidence of GSD-Ia in the population is reported to be 1 in 100,000, with a carrier frequency of ~1 in 150 (2). To date, 86 disease-causing mutations in *G6PC* have been identified (6), including missense mutations, nonsense mutations, frameshifts resulting from insertions and deletions, and rare gene rearrangements (6), with missense mutations being the

Correspondence to: Dr Xiaou Shan, Department of Pediatrics, The Second Affiliated Hospital of Wenzhou Medical University, 109 West Xueyuan Road, Wenzhou, Zhejiang 325027, P.R. China
E-mail: sssxooo@sina.com

Dr Zi-Bing Jin, Division of Ophthalmic Genetics, Laboratory for Stem Cell and Retinal Regeneration, The Eye Hospital of Wenzhou Medical University, 270 West Xueyuan Road, Wenzhou, Zhejiang 325027, P.R. China
E-mail: jinzb@mail.ey.ac.cn

*Contributed equally

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most common (6). Although GSD-Ia is a pan-ethnic disease, the incidence of specific mutations varies between ethnic populations (4). For example, the c.648G>T mutation is prevalent in East Asians (4), whereas c.247C>T is common in the Jewish population (6). *G6PC* mutations may be classified into three categories based on their predicted topological position: Non-helical, helical and active site. Typically, non-helical mutations have a lesser effect on enzymatic activity compared with helical mutations (8), and *in vitro* experiments have demonstrated that *G6PC* containing non-helical mutations may retain enzymatic activity (9). However, the precise association between mutations and disease severity remains to be fully elucidated. In the present study, a missense mutation of *G6PC* was identified in a 3-month-old female patient from a consanguineous family. The association between the *G6PC* genotype and disease phenotype in this patient is in contrast to that previously described (8). The results of the present study suggested that the prevailing view that mutations in non-helical regions of *G6PC* result only in mild phenotypic manifestations may require revision.

Materials and methods

Patient characteristics. The 3-month-old female patient with hypoglycemia, hepatocellular dysfunction and lactic acidosis was groaning frequently. The patient was in a clear state of mind and not convulsing. The parents brought her to The Second Affiliated Hospital of Wenzhou Medical University (Wenzhou, China), for an emergency visit, following which she was admitted to the Department of Pediatrics. She weighed 6 kg and was 60 cm. The patient did not suffer from severe recurrent infections. Her parents are first cousins (Fig. 1A). There was no history of note. A comprehensive examination of the patient was performed, including a physical examination, vein blood gas analysis, an electrolyte test, an abdominal sonography and biochemical analyses.

Molecular screening. Informed consent was obtained from everyone involved in the present study. The study protocol was approved by The Second Affiliated Hospital of Wenzhou Medical University Ethics Committee (Wenzhou, China). Genomic DNA was isolated from peripheral blood samples using a genomic DNA extraction kit (RelaxGene Blood DNA system; Tiangen Biotech Co., Ltd., Beijing, China), according to the manufacturer's protocols. The five exons and flanking introns of the *G6PC* gene were amplified by polymerase chain reaction (PCR) with forward, AGTCCACTTCGAACAGCCAG and reverse, CCGATCGCGTCATGGAAAAC primers. The 50 μ l total reaction volume contained 0.4 μ mol/l of each primer, 0.2 mmol/l dNTPs, 1X PCR buffer, 50 ng genomic DNA, and 1 unit Taq DNA polymerase (Takara Biotechnology Co., Ltd., Dalian, China). The PCR cycling conditions consisted of denaturation at 95°C for 4 min followed by 40 thermal cycles of 95°C for 30 sec, 58°C for 30 sec, and 72°C for 42 sec. Resulting PCR products were electrophoresed on a 1.5% agarose gel and purified using a PCR purification kit (AxyPrep PCR Cleanup kit; Hangzhou Axygen Biotechnology Ltd., Hangzhou, China). The PCR products were sequenced using an automated sequencer (ABI Prism 3100 Genetic Analyzer; Applied Biosystems; Thermo

Fisher Scientific, Inc., Waltham, USA), and compared with a reference sequence. The gene sequences surrounding the mutation site in various species were obtained from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/pubmed/) and analyzed to determine whether the mutation site was conserved.

Results

Clinical examination. The 3-month-old female patient was born in a consanguineous family (Fig. 1A). She was groaning frequently. There was no history of note. Physical examination revealed shortness of breath, symmetrical rough breath sounds, regular respiratory rhythm, and abdominal distension with enlarged liver (7 cm below the right costal margin). The spleen was not palpable. An increased level of lactic acid revealed that the patient had lactic acidosis. Vein blood gas analysis revealed that pH, PCO₂ and HCO₃⁻ levels were below normal, and that the absolute value of base excess was increased, suggesting metabolic acidosis (Table I). In addition, the shortness of breath exhibited by the patient indicated metabolic acidosis. A fasting blood glucose level of 2.73 mmol/l indicated that the infant suffered from severe hypoglycemia. Furthermore, increased levels of alanine aminotransferase, aspartate aminotransferase, and γ -glutamyl transpeptidase suggested liver dysfunction. Additionally, the levels of uric acid and triglycerides were also increased (Table II). Abdominal sonography identified hepatomegaly with enhanced echo texture (Fig. 1B) and nephromegaly (Fig. 1C), with the size of left kidney measured at 72x40 mm and the size of right kidney measured at 71x30 mm. The enlarged organs indicated the severity of the disease. A viral hepatitis serology screen revealed that the patient was positive for hepatitis B surface antigen (461.5), negative for hepatitis B core antibody, and that total immunoglobulin was normal. The patient was negative for human immunodeficiency virus. In summary, the patient's clinical manifestations and test results suggested GSD-Ia.

Diagnosis of GSD-Ia. Gene sequencing revealed that the patient was homozygous for a missense mutation in the *G6PC* gene, c.518T>C (p.L173P; Fig. 2A), which changed the codon for leucine at position 518 to a codon for proline. Pedigree analysis revealed that the patient's father and mother are heterozygous for this mutation (Fig. 1A). By comparing the *G6PC* sequence across various species, it was determined that the mutation is located in a highly conserved region of the *G6PC* gene (Fig. 2B). Furthermore, the p.L173P mutation is absent from the 1000G database (<http://www.1000genomes.org/>), suggesting it is a very rare mutation. This finding, together with the severe symptoms of the patient, suggested that there is a high probability that the p.L173P mutation results in abnormal *G6PC* enzyme function. However, this mutation affects a non-helical region of the protein, which previous studies suggest are of less importance compared with the helical regions for enzyme function (8). Therefore, the phenotypic characteristics associated with a mutation in the non-helical region of the protein are expected to be mild. However, in the present study the clinical manifestations were severe.

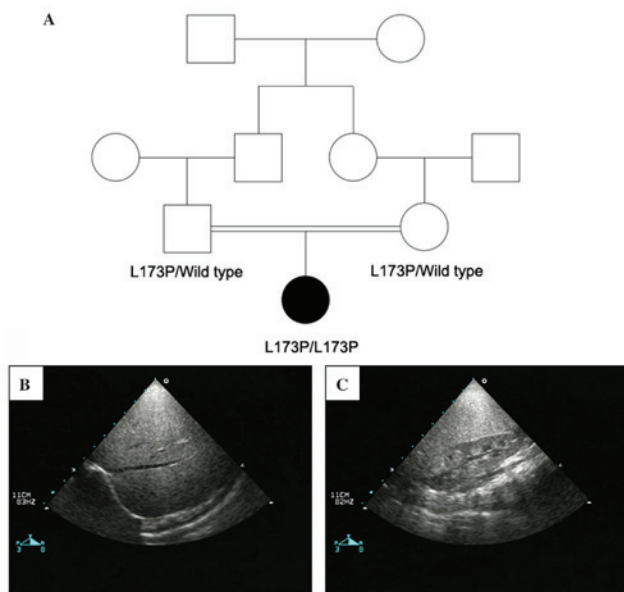


Figure 1. (A) Pedigree analysis. The patient is demarcated in black. The patient was homozygous for the mutation and her parents were heterozygous carriers. (B) Liver sonography revealed enlargement of the liver. (C) Kidney sonography revealed enlargement of the kidneys.

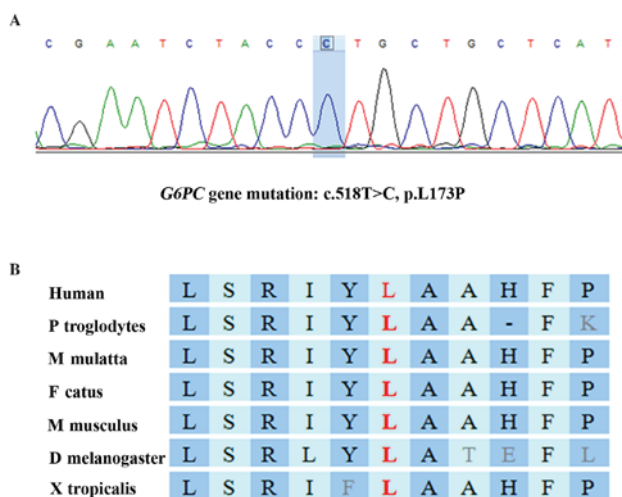


Figure 2. Sequence analysis of the *G6PC* gene. (A) Gene sequencing identified a homozygous missense mutation in the *G6PC* gene of the patient: c.518T>C, p.L173P. (B) Amino acid alignment of the region of *G6PC* surrounding the patient's mutation site with the analogous region in various species. G6PC, glucose-6-phosphatase- α ; L, leucine; P, proline.

Discussion

G6PC is a 357-amino acid glycoprotein anchored to the ER membrane by nine transmembrane helices, with an enzymatic active site facing the ER lumen (8). The amino-terminus of G6PC is located in the ER lumen, and the carboxyl-terminus is in the cytoplasm (4,8,10). The *G6PC* gene, located on chromosome 17q21, is a single copy gene composed of five exons (10). The only known cause of GSD-Ia is mutation of *G6PC*. Mutations have been divided into three categories, non-helical, helical and active site, based on their predicted topological position. In general, non-helical mutations are

Table I. Results of patient vein blood gas analysis.

Parameter	Test value	Change	Reference range
pH	7.26	↓	7.35-7.45
PCO ₂ , mmHg	24.0	↓	35-45
HCO ₃ , mmol/l	10.3	↓	22-27
BE, mmol/l	-15.1	↑	-2-3

BE, base excess.

Table II. Results of patient blood biochemical analysis.

Parameter	Test value	Change	Reference range
FBG, mmol/l	2.73	↓	3.90-6.10
Lactic acid, mmol/l	12.20	↑	0.6-2.20
ALT, U/l	102	↑	7-40
AST, U/l	169	↑	13-35
GGT, U/l	820	↑	7-38
UA, μ mol/l	1035	↑	89-357
TG, mmol/l	7.90	↑	0.56-1.70

FBG, fasting blood glucose; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ -glutamyl transpeptidase; UA, uric acid; TG, triglyceride.

thought to have a smaller effect on enzyme activity compared with those in transmembrane helices (8). In the present study, the case of a 3-month-old infant with a c.518T>C (p.L173P) non-helical *G6PC* mutation is reported. According to the prevailing view, the infant would be expected to have mild phenotypic characteristics. However, the clinical manifestations were clear, as the patient suffered from severe hypoglycemia and hepatomegaly. Abdominal sonography revealed nephromegaly. Li *et al* (11) reported a 3-year-old girl who was not diagnosed with GSD-Ia until the age of 2. The phenotypic characteristics of this patient were fasting hypoglycemia, hepatomegaly, hypercholesterolemia and recurrent epistaxis. The patient did not suffer from nephromegaly or metabolic acidosis. Her biochemical results were improved through a diet of regular uncooked cornstarch and frequent carbohydrate feeds. That patient was not born of a consanguineous union. DNA sequencing identified that the mother had a heterozygous R83H (G327A) mutation and the father had a heterozygous L173P (T597C) mutation (11). Thus, the patient had a p.L173P mutation, as in the present study. While the two girls suffered from identical mutations, the severity of their phenotypic characteristics was very different. The results of the present study suggested that non-helical mutations may result in severe clinical manifestations, and that phenotypic heterogeneity exists in p.L173P *G6PC* mutations.

GSD-Ia is a pan-ethnic disorder; however, the incidence of mutations varies between ethnicities. To date, no reports of a p.L173P mutation have been presented in any ethnicity other than Chinese. This may indicate that the incidence of the p.L173P mutation is relatively high in the Chinese population.

In the present study, hypoglycemia and lactic acidosis were under control following administration of intravenous glucose to the infant at a dose of 6 mg/kg/min. Nocturnal nasogastric infusion of glucose and intermittent oral administration of uncooked cornstarch are the primary dietary therapies for GSD-Ia (12). However, even with dietary therapy, long-term clinical problems may occur. These include growth retardation, hepatomegaly, intermittent hypoglycemia, lactic acidemia, hyperlipidemia, adenoma, gout associated with hyperuricemia, proteinuria and nephrolithiasis with progressive renal failure. Replacement therapy is a promising alternative strategy for GSD-Ia treatment, and may involve administration of G6PC, liver-kidney transplantation or gene therapy. As G6PC is hydrophobic, it is difficult to isolate in an active, soluble form that would facilitate its administration to patients (5). Case reports have revealed that liver-kidney transplantation may rectify the metabolic disorder associated with GSD-Ia (13,14). However, liver-kidney transplantation brings with it associated risks and challenges. Gene therapy, while not yet developed for the clinic, may in the future provide a strategy for the treatment of GSD-Ia (15,16). Previous studies have demonstrated the efficacy of gene therapy in treating mouse models of GSD-Ia (15,17,18). In addition, alternative therapies, including bone marrow-derived myelomonocyte transplantation, may have the potential to restore normal liver function (19).

In conclusion, the present study reports the case of a 3-month-old female Chinese patient with GSD-Ia and identified a *G6PC* missense mutation of c.518T>C (p.L173P) located in a highly conserved area. The severity of the phenotype was inconsistent with that predicted by her genotype. The incidence of the p.L173P mutation may be relatively high in the Chinese population. Knowledge of the various phenotypic presentations of the p.L173P mutation may be beneficial for future investigations.

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