Male inheritance of X-linked liver glycogenosis from an undiagnosed maternal grandfather in a Chinese pedigree: a report of two cases

Ping Li, Tao Xu, Qingqing Lu, Jianqi Liang, Zhen Zhang, Yu Fang, Xiaobing Xie

Abstract

Hepatic phosphorylase kinase (PhK) plays an important role in glycogen metabolism by activating phosphorylase. Patients with PhK deficiency may get glycogen storage disease (GSD) type-Ixa, an X-linked liver glycogenosis disease. To inform genetic counseling in a family with two affected GSD brothers, we performed a genetic analysis. The GSD in the older brother was confirmed by histological examination of a liver biopsy, which showed glycogen accumulation in liver cells. A liver biopsy was not available from the younger brother. The two patients and their parents were analyzed by whole exome sequencing. A pathogenic mutation in a gene encoding a regulatory subunit of PhK, PHKA2 located on chromosome Xp22, was identified as c.G3373A (p.E1125K) and confirmed by Sanger sequencing. The proband’s maternal grandparents and the brothers and sisters of the proband’s maternal grandfather were physically examined and genetically tested by Sanger sequencing. Pedigree analysis showed that the mother was a carrier and that the two patients inherited the mutation from their undiagnosed maternal grandfather. Moreover, among the maternal grandfather and four granduncles, three of them possessed the same mutation and four suffered from fatty liver. This is the first report of this mutation causing X-linked liver glycogenosis in a Chinese family and shows that GSD IXa is a mild form of glycogenosis in terms of clinical symptoms, indicating that GSD may be underdiagnosed or underestimated. Nevertheless, to provide appropriate intervention and genetic counseling, early identification of the genetic cause is imperative. This study was approved by the Ethics Committee of First Affiliated Hospital, Hunan University of Chinese Medicine (approval No. HN-LL-ZFKY-2018-001-01) on January 12, 2018.

Keywords: case report, genetic mutation, glycogen storage disease, hepatic phosphorylase kinase, whole exome sequencing

Introduction

Congenitally abnormal glycogen metabolism leads to glycogen storage disease (GSD), which is a group of inherited metabolic disorders due to enzymes deficiency in the pathway of glycogen synthesis and decomposition. The estimated incidence of GSD is 1 in 20,000 to 43,000 live births.[1] GSD is a heterogeneous group of over 14 rare hereditary diseases that affect glycogen storage in the liver. GSD type VI and IX are caused by deficiency of hepatic phosphorylase,[2] the rate limiting enzyme of glycogenolysis. Hepatic phosphorylase is activated by a series of enzymatic reactions involving phosphorylase kinase (PhK) and cAMP-dependent protein kinase. PhK is a hexadecane enzyme complex composed of α, β, γ and δ subunits, which are encoded by different genes. The δ subunit is calmodulin, which together with phosphorylation of the α and β subunits modulates the activity of the catalytic γ subunit.[3-4] PhK phosphorylates and activates glycogen phosphorylase in the glycogen decomposition cascade.[5]

PHKA2 encodes the liver subtype of the PhK α subunit, and PHKA2 mutations can lead to PhK enzyme deficiency and the X-linked disease, X-linked liver glycogenosis (XLG) or GSD IXa.[4,6] XLG is often a benign GSD disease and is one of the most common forms of GSD. However, its main characteristics during childhood are growth retardation, abnormal liver functions, hepatomegaly, and high levels of alanine transaminase (ALT), aspartate aminotransferase (AST), cholesterol and triglycerides.[3,7] These symptoms gradually alleviate with age, and adults often become asymptomatic,[8] which is different from other types of GSD. The clinical symptoms of two XLG subtypes, XLG-I and XLG-II, are very similar. PhK activity in hepatocytes of both subtypes is reduced and PhK activity is deficient in peripheral blood cells of patients with XLG-I; however, PhK activity in leukocytes and erythrocytes of XLG-II patients may be normal or even elevated.[9] PHKA2 mutations have been described in patients from Japan, Korea and some western countries.[3,8,9] Here, we describe two cases in a Chinese family with XLG-I.

Cases report

Clinical history and observations

The first patient (the proband, male), 10 years old, was born by Caesarean section to non-consanguineous parents in January
2010. At birth he weighed 3400 g and was 50 cm long. At 2 years and 7 months he developed an abdominal distention. Physical examination revealed marked enlargement of the liver that was 5.1 cm below the right costal margin and raised levels of liver enzymes. The value of AST is 126.83U/L (reference range: 0–40U/L), and the value of ALT is 50.90IU/L (reference range: 0–41IU/L). Furthermore, his height development was lower than normal of same age. His mother stated that he had no family history of hepatitis. Oral armillarisin solution and bifendate had little effect. A liver aspiration biopsy and histological examination by light and electron microscopy revealed that his liver parenchyma cells had ballooning changes, eccentric nuclei, PAS-positive cytoplasm, and a foamy appearance (Fig. 1). Glycogen storage disease was therefore diagnosed. His signs and symptoms have not substantially improved since the onset of the disease.

Physical and biochemical examinations performed in October 2016 showed the liver spanning 3.4 cm below the right costal margin and AST 117U/L, ALT 129U/L, and fasting blood sugar (FBS) 3.84mM.

The second patient (male), 8 years old, was born by Caesarean section in October 2011. At birth he weighed 3000 g and was 50 cm long. At 2 years of age he suffered from the same elevated liver enzyme and hepatomegaly symptoms as his brother (Patient 1). Physical and biochemical examinations performed in October 2016 showed his liver to be 3.3 cm below the right costal margin and AST 98U/L, ALT 102U/L, and FBS 3.76mmol/L. We did not perform a liver biopsy.

**Pedigree and family history**

The patients’ mother was born in 1983 and had no obvious abnormality on physical examination. The patients’ maternal grandfather was born in 1954. His height was 159 cm, which is lower than the average height of a Chinese adult man. He claimed no liver disease since childhood, and he had not been tested in hospital. In order to find the cause of disease and facilitate subsequent family investigation, some members of this family were recently advised to undergo blood biochemical examination. The patients’ grandfather has two sisters and five brothers. All five brothers underwent physical examination, blood biochemistry evaluation and ultrasonography of the abdomen. The results showed their body heights were between 156.5 cm and 168.5 cm, and their blood biochemistry results were roughly normal, with only mildly abnormal bilirubin and blood lipid levels. Four of five brothers had different degrees of fatty liver. The examination results of these five brothers are shown in Table 1.

The two sisters of the patients’ grandfather reported no hepatic inadequacy or other symptoms. Nevertheless, they were not willing undergo examination. We advised all other family members to undergo blood biochemistry analysis genetic testing. The family tree is presented in Figure 2.

This study was approved by the Ethics committee of First Affiliated Hospital, Hunan University of Chinese Medicine (approval No. HN-LL-ZFKY-2018-001-01) on January 12, 2018 and conducted in accordance with the Declaration of Helsinki. The patients’ guardians signed the written informed consent before enrollment of the patients.

**Sequencing analysis**

Blood samples were collected from the 11 subjects indicated with an asterisk in Figure 2, including the two patients, their parents, uncle, maternal grandmother and maternal grandfather and granduncles. Whole exome sequencing was conducted on four of these samples, including both patients and their parents, by high-throughput sequencing analysis using the standard Illumina HiSeq X-Ten pipeline. Whole exome sequencing revealed a single point mutation, c.G3373A in exon 32 of PHKA2 located on Xp22, which caused amino acid residue 1125 of PHKA2 (NM_000292) to change from glutamate to lysine (p.E1125K). This mutation was present in both patients, while their mother was a heterozygous carrier and their father did not carry the mutation. Sanger sequencing using an ABI 3730XL was conducted to determine the presence of this mutation in 11 family members including relatives of the patients’ mother. The patients’ grandfather and two granduncles had the same mutation, while the patients’ grandmother, uncle (mother’s brother) and father did not have the mutation. Because of the genetic characteristics of X-linked recessive genetic diseases, the aunt and her daughter are likely to be carriers of the mutation but we were unable to obtain their blood samples and sequence their DNA.

![Figure 1](image.png)

**Figure 1.** Liver histology of Patient 1 shows diffuse clear cytoplasm in hepatocytes and hepatocyte ballooning (red arrow). (A) Light microscopy; (B) scanning electron microscopy (KYKY-EM6200, China). Original magnification: 40× and 400× in A and B, respectively.
**Subtyping for XLG**

XLG can be diagnosed through the above analysis. However, according to different PhK activity in leukocytes and erythrocytes, XLG can be classified as XLG-I and XLG-II.[13] The ultimate diagnosis of the two patients was XLG-I because the p.E1125K mutation in PHKA2 was previously confirmed to be the cause of the XLG-I subtype.[19]

**Treatment and prognosis for XLG**

After the two patients were diagnosed as GSD by genetic analysis in March 2017, they were administered uncooked cornstarch at 2.2 g/kg, three times/day as adjuvant therapy when Patient 1 was 7 years 2 months old and Patient 2 was 5 years and 5 months old. The two patients were also instructed to reduce monosaccharide intake to alleviate glycogen accumulation in the liver. After taking uncooked cornstarch for 1 year, their blood biochemistry values of liver function returned to normal. After taking uncooked cornstarch for 1.5 years, their liver shapes and sizes returned to normal as determined by abdominal B-ultrasound. Thereafter, the patients’ height and weight were regularly measured and blood tests and abdominal B-ultrasounds were regularly performed. These results are listed in Table 2.

**Discussion**

GSD can be subdivided into different subgroups according to enzyme defects in the glycogen metabolism pathway [1,10]. These subgroups also show physiological differences.[11] Clinical manifestations are generally associated with liver and muscle because glycogen is accumulated in these organs.[12] GSD type I, III, VI and IX normally involve the liver,[13] and GSD type I is the most serious because it affects gluconeogenesis and glycolysis.[11]

GSD-IX is caused by functional deficiency of liver phosphorylase systems and its clinical manifestations are growth retardation, fasting ketoacidosis, and hepatomegaly due to abnormal material metabolism. GSD-IXa is caused by functional deficiency of α subunit of PhK and inability to activate the phosphorylase.

PHKA1 encodes subunit α of PhK, pathogenic variants in PHKA1 can cause the rare X-linked disorder muscle PhK deficiency. PHKA2 also encodes subunit α, while pathogenic variants in PHKA2 can cause the most common form, liver PhK deficiency (XLG). [14] The mutation p.E1125K in PHKA2, is a pathogenic mutation of XLG and is registered in the human gene mutation database.[13] GSD IXa has been further divided into types IXa1 (GSD9A1), with no PhK activity in liver or erythrocytes, and IXa2 (GSD9A2), with no PhK activity in liver but normal activity in erythrocytes. Patients with the mutation p. E1125K in PHKA2 are considered type IXa1. However, we did not validate PhK activity in either liver or peripheral blood in the two patients of this study.

Both patients in this study presented symptoms of growth retardation, abnormal liver function and hepatomegaly; however, they did not show symptoms that are found in the vast majority of the other types of GSD, such as metabolic acidosis and hypoglycemia. Most cases of XLG show benign symptoms, which gradually weaken with age.[6,10,15] The initial growth retardation occurs between 2–10 years of age, and these patients usually attain normal height as adults. A longitudinal study of 41 patients showed that hepatomegaly occurred in 92%, growth retardation in 68%, and elevation of glutamate pyruvate transaminase in 56% of patients.[14] In addition, patients outgrow their clinical symptoms, and even become asymptomatic in adulthood. Therefore, a clinical diagnosis may be difficult to obtain for some patients with XLG. Nevertheless, the disease can be passed on from undiagnosed males to their offspring. To date,
Table 2

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no large-scale studies have assessed the incidence and prevalence of XLG.

GSD-Ixa and other X-linked recessive disorders are only symptomatic in males.[16] Reports of females with an X-linked disorder are seldom seen. The same situation occurred in this family; the disease was inherited by the two patients from their grandfather, and their mother was a heterozygous carrier without any symptoms. In the pedigree, the four brothers of the patients’ grandfather all suffered various degrees of liver fatty, and the patients’ grandfather (pedigree number: II4) had mildly abnormal blood sugar and lipid levels. The genetic analysis indicated that the grandfather and granduncles (pedigree numbers: II2, II4 and II6) were probably all XLG patients, although they were not diagnosed in adolescence. The two sisters of the grandfather have a 50% chance of being heterozygous carriers; however, they did not consent to genetic testing. The genetic analysis of this pedigree made prenatal diagnosis of offspring and genetic counseling very useful for the family.

From March 2017, the two patients were given uncooked cornstarch and their dysfunction of liver and hepatomegaly symptoms gradually returned to normal. We therefore suggested that patients diagnosed with XLG should be treated with uncooked cornstarch as early as possible. However, it should be noted that due to lack of amylase, undercooked starch is usually not effective for children under 0.5 to 1 years of age. According to the biochemical results of these two patients, their liver enzymes returned to normal in less than a year after taking uncooked cornstarch and this was the first time that their liver enzymes have returned to normal since they were found to have XLG. And in October 2018, the abdominal ultrasound results of these two patients showed normal for the first time. However, an abdominal ultrasound in 2019 showed that the livers of these two patients were slightly enlarged, which may be related to the irregular use of uncooked cornstarch at that time. Then in 2020, their results of abdominal ultrasound showed normal.

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Author contributions

PL and XX contributed to study concept and design. TX, JL, and YF provided samples and materials. PL and ZZ participated in data acquisition. PL and TX analyzed data. PL, QL, and XX contributed to manuscript writing and critical revision. All authors approved the final version of the manuscript.

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Declaration of patient consent

The patients’ guardians received information about the study, and signed the written informed consent, in which they have given their consent for the patients’ clinical information to be reported in the journal. They understand that the patients’ names and initials will not be published and due efforts will be made to conceal their identity.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References