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Case Report

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Two Cases of X-linked Liver Glycogenosis in Hunan Province: Transmission from the Undiagnosed Maternal Grandfather

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Abstract

X-linked liver glycogenosis (XLG), also known as glycogen storage disease (GSD) type-IXa, is characterized by hepatomegaly, abnormal liver functions and growth retardation. This disease is a result of a deficiency of hepatic phosphorylase kinase (PHK), which plays an important role in glycogen metabolism by activating phosphorylase. We aim at identifying the genetic cause of the GSD running in a family with two affected boys, and hence develop proper management and genetic counseling. The boys presented with typical GSD signs and symptoms. The histology of the liver biopsy from the old brother showed glycogen accumulation in hepatocytes and confirmed his condition of GSD. The younger brother did not have a biopsy. Whole exome sequencing was used to analyze the genetic structure of the proband patients and their parents. Sanger sequencing was used to validate and confirm the identified mutation. The results showed that there was a known pathogenic mutation (p.E1125K) in *PHKA2* gene located on chromosome Xp22, which encodes the regulatory subunit of PHK. Pedigree analysis revealed that the mother was a carrier, and the disease of both brothers was transmitted through their undiagnosed maternal grandfather. This is the first report of XLG caused by this mutation in China, and it indicated that GSD may be undiagnosed or underestimated since GSD IXa is one of the mild form of glycogenosis in terms of clinical symptoms. However, it is necessary to identify the genetic cause in order to perform effective intervention and genetic counseling.

Keywords: X-linked liver glycogenosis (XLG); Glycogen storage disease (GSD); Hepatic phosphorylase kinase (*PHKA2*); Whole exome sequencing (WES); Genetic mutation

Introduction

The glycogen storage disease (GSD) is caused by the inborn errors of glycogen metabolism. It is due to the deficient hepatic phosphorylase activity and presents as a heterogeneous group of liver glycogenosis. According to the lack of the enzymes, GSD can be divided into at least 16 types.

The phosphorylase, the rate limiting enzyme of glycogenolysis, is activated by a cascade of enzymatic reactions related to protein kinase and phosphorylase kinase (PHK). PHK plays an important regulatory role in glycogen phosphorylase for the degradation of glycogen to glucose-1-phosphate. It is a complex enzyme which consists of four different subunits α, β, γ and δ. These subunits together form a hexadecameric structure $(\alpha, \beta, \gamma, \delta)_4$. The γ subunit harbors the site for the catalytic activity which is regulated by the phosphorylation of the α and β subunit and by the interaction with the δ subunit, which is a calmodulin for Ca^{2+} regulation [1]. These subunits are encoded by different genes. The PHKA1 and *PHKA2* genes are located on Xp13 and Xp22 and encode for the muscle and liver isoforms of the α subunit of PHK, respectively.

The X-linked liver glycogenosis (XLG), a common type of GSD, also known as GSD IXa, is caused by enzymatic deficiency of PHK [2,3] due to the pathogenic mutations in *PHKA2*. Unlike other types of GSD, XLG is generally a benign disease, mainly characterized by hepatomegaly, growth retardation and abnormal liver function during childhood. The clinical symptoms gradually improve with age, and patients are often asymptomatic in adulthood. XLG can be divided into two subtypes: XLG-I and XLG-Ⅱ. Both types of patients show very similar clinical symptoms. However, patients with XLG-I show clear *in vitro* enzyme deficiency of PHK in erythrocytes, leukocytes, and liver, whereas, XLG-Ⅱ patients show normal or even elevated *in vitro* PHK enzyme activity in erythrocytes and leukocytes and varying activity in liver. Both types of XLG have been shown to be caused by mutations in the *PHKA2* gene. Mutations of *PHKA2* have been reported in some western countries, Korea, and Japan [4-6]. In this report, we present two cases in a family with XLG-I in China.

Case Presentation

Clinical history and observations

The first patient was born in Jan, 2010 to unrelated parents, with a caesarean birth weight of 3400 g and length of 50 cm. At age of 2 years 7 months, he was noted to have an abdominal distention. Physical examination revealed growth retardation, marked hepatomegaly, with a liver span of 5.1 cm below the right costal margin, and high level of liver enzymes (AST: 126.83U/L, ALT:50.90IU/L). His mother claimed no family history of hepatitis. The boy was given armillarisin oral solution and bifendate pills. However, there was poor efficacy. Therefore, a needle biopsy of the liver was taken. Light and electron microscopy showed ballooning of liver parenchyma cells, which had eccentric nuclei and foamy, PAS-positive cytoplasm (Figure 1). Liver histology and electron microscopy confirmed the diagnosis of glycogen storage disease. From the onset to the present, his symptoms and signs have not been significantly improved. The recent physical examination

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showed that body height was 110 cm, weight was 22 kg, AST 78 U/L and ALT 101 U/L, a liver span of 3.4 cm below the right costal margin.

The second patient was born in October 2011 with a caesarean birth weight of 3000 g and length of 50 cm. Unfortunately, he was suffered from the same symptoms such as hepatomegaly, elevated liver enzymes at the age of 2 years just like his brother (case 1). Recent physical examination showed that body height was 103 cm, weight was 21 kg, AST 98 U/L and ALT 102 U/L., a liver span of 3.3 cm below the right costal margin. No liver biopsy has been performed.

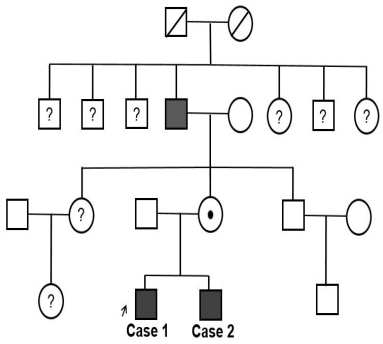

Figure 1: Pedigree of the family. Two proband patients and their parents, grandparents, and uncle were available for genomic analysis. Grandfather was with undiagnosed phenotype. Arrow refers to the proband; ○=female; □=male; ⊙=obligate female carrier; ■=patient (case); ⊘=deceased; ?=no genetic analysis.

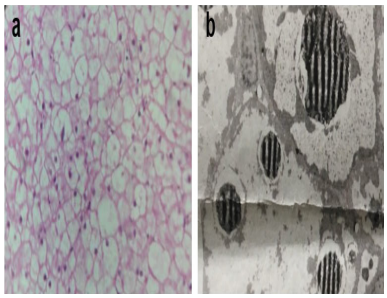

Figure 2: Liver histology of Patient 1 shows diffuse clear cytoplasm in the hepatocytes and hepatocyte ballooning change (a: Light microscopy × 40; b: Eccentric microscopy × 400).

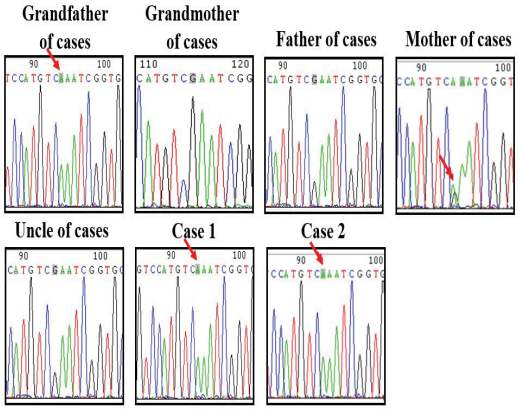

Figure 3: Sanger sequencing of the PHKA2 gene in the family. Arrows refer to the mutation site of p.E1125k in exon 33 of the gene of the samples. Patient 1, Patient 2 and the grandfather are all homozygous for the mutation, while the mother of patients is a heterozygous carrier with normal phenotype. The father and the uncle of cases are wild type for this mutation.

Pedigree and family history

The boys' maternal grandfather was born in 1954. His present body length is 159 cm, which is below the average range for the Chinese population. He claimed he had no liver disease from the childhood up to now. He was told to take the blood biochemical examination recently, and the results showed that the liver function was normal, with only mild increase in total cholesterol.

The grandfather has five brothers and two sisters. All of the brothers had different degrees of cirrhosis and liver dysfunction. Both of the sisters claimed that they had no dysfunction of liver and other symptoms. In addition, we are assisting all of the family members with blood biochemistry and genetic analysis.

A general overview of the family's pedigree is presented in Figure 2.

Sequencing analysis

Four (4) blood samples, including the two patients and their parents, were collected. WES was performed on these samples using Illumina HiSeq X-Ten instrument and the high throughput sequencing analysis were proceeded using standard pipeline. WES revealed a single point mutation, changing the glutamate at protein position 1125 to lysine (p.E1125K) in *PHKA2* gene (NM_000292) located on Xp22. This mutation was found in both of the patients and their mother, who was a heterozygous carrier, while the father was normal (no mutation). Sanger sequencing was performed using ABI 3730XL on three (3) samples and validated the mutation in the family members including the boys' grandparents. As expected, the boys' grandfather had the same mutation (Figure 3). While the boys' father, grandmother and the uncle (mother's brother) were normal. Unfortunately, the aunt and her daughter are not available for DNA sequence analysis although they have a great chance of becoming carriers because of the genetic characteristics of X linked recessive genetic disease.

Subtyping for XLG

From the above analysis, the diagnosis of XLG can be given. However, since that XLG can be divided into two subtypes based on the PHK activity in the erythrocytes and leukocytes, we have to go further in the diagnosis. The mutation p.E1125K in *PHKA2* was previously validated as the cause of the XLG-I subtype [4]. Therefore, the final diagnosis of the two patients is XLG-I.

Discussion

The medical problem of GSD is sub-classified according to respective enzymatic defects along the pathway of glycogen metabolism [7-9]. Because glycogen is mainly accumulated in liver and muscle, clinical manifestations are typically related with these organs [10]. GSD-IX is due to the functional deficiency of liver phosphorylase systems and is clinically characterized by hepatomegaly, fasting ketoacidosis, and growth retardation. GSD-IXa is caused by the functional deficiency of the α subunit of phosphorylase kinase (PHK), which activates the phosphorylase. The mutation in *PHKA2* gene encoded PHK could lead to GSD-IXa, or XLG. The mutation p.E1125K in *PHKA2* was validated as a pathogenic mutation site for XLG by Jan Hendrickx in 1999 [7], and have been registered in the human gene mutation database (HGMD).

Both of the patients in this study presented with hepatomegaly, abnormal liver function and growth retardation, but no evidence of hypoglycemia and metabolic acidosis, which are frequently observed

in most of the other types of GSD. Most of the XLG cases show benign symptoms which are attenuating as the children grow up [9,11,12]. Initial growth retardation has been noted between the ages of 2-10 years, and these patients often approach normal height in adulthood. Furthermore, clinical symptoms gradually disappear with age, leaving most adults asymptomatic. Therefore, for some patients with XLG, it may not be diagnosed timely or it is difficult to reach a clinical diagnosis. However, the disease could pass to the offspring through undiagnosed males. At present, no large-scale study assessing the incidence and prevalence of XLG has been reported.

Conclusion

The X-linked recessive disorder, like GSD-IXa, usually produces symptomatic disease only in male [13]. The previous reports of female with X-linked disorder are still rare in literature. It is the same situation as in this family: the two boys' disease is inherited from their grandfather, and the mother of the cases is a heterozygous carrier with normal phenotype. In the pedigree, the five brothers of the grandfather all suffered various degrees of cirrhosis and liver dysfunction. That indicated they are all probably to be patients of XLG, and the two sisters may be possible heterozygous carriers, even though their *PHKA2* gene are not analyzed. The current genetic and pedigree analysis made it valuable for the offspring of the family to do a prenatal diagnosis and genetic counseling.

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Contributors

Study concept and design: Ping Li, Xiaobing Xie; Samples and materials: Tao Xu, Yu Fang; Data acquisition: Ping Li, Zhen Zhang; Data analysis: Ping Li, Yi Lao; Manuscript writing and critical revision: Ping Li, Xiaobing Xie.

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Competing Interests

None declared.

Patient Consent

Patient consent is obtained.

Ethics Approval

This study was approved by the Ethics Committee of First Affiliated Hospital, Hunan University of Chinese Medicine.

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