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Brief Communication

Novel mutations in PHKA2 gene in glycogen storage disease type IX patients from Hong Kong, China

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ABSTRACT

The diagnosis of glycogen storage disease (GSD) type IX is often complicated by the complexity of the phosphorylase kinase enzyme (PHK), and molecular analysis is the preferred way to provide definitive diagnosis. Here we reported two novel mutations found in two GSD type IX patients with different residual enzyme activities from Hong Kong, China using genetic analysis and, provided the molecular interpretation of the deficient PHK activity. These two newly described mutations would be useful for the study of future GSD patients.

1. Introduction

Glycogen storage disease (GSD) type IX results from a deficiency of phosphorylase kinase (PHK; EC 2.7.1.38) [1]. This enzyme plays a significant role in the control of the breakdown of glycogen to glucose. PHK consists of four different subunits, α, β, γ and δ. α- and β-subunits have regulatory functions. The γ-subunit has catalytic function and δ-subunits has Ca^{2+} binding functions [2]. There are two a-subunit isoforms, muscle and liver, which encode from the X-linked genes PHKA1 and PHKA2 on Xq12-q13 and Xp22.2-p22.1 respectively [3]. Defects in liver isoform (PHKA2) give rise to X-linked glycogenosis (XLG), characterized by hypoglycaemia, hepatomegaly, chronic liver disease, growth retardation and delayed motor development, hypercholesterolaemia, hypertriglyceridaemia, and hyperketosis following fasting [4]. XLG is further subclassified into XLG1 with no detectable activity of phosphorylase kinase in liver and erythrocytes and XLG2 with normal activity in erythrocytes but deficiency was only observed in liver. These symptoms often improve with age and may even normalize in adulthood [5].

The diagnosis of glycogen storage disease type IX is often complicated by the complexity of the phosphorylase kinase enzyme. In some cases, the measurement of the enzymatic activity of PHK in blood cannot provide a definite diagnosis and the patients may still need to undergo invasive diagnostic investigations such as a liver biopsy [6]. Molecular analysis is important to establish a definitive diagnosis and in some cases, it may also avoid from further invasive procedures. The genetic diagnosis is also essential in genetic counseling of the patients and their extend family as it differentiates the X-linked inheritance from autosomal recessive inheritance of other types of GSDs. Analysis of the X-linked PHKA2 gene in GSD type IX has been evaluated in more than 40 families [6–9]. Here, we reported investigation of two GSD type IX patients with deficiency of PHK from Hong Kong, China. We found two novel mutations in the PHKA2 gene which expand the spectrum of mutations in this gene. Based on the mutations, we provide the molecular interpretation of the phosphorylase kinase defect.

2. Case Report

Patient 1 was a 3 year-old boy who was the first child in his family. He was born at term after an uneventful pregnancy to non consanguineous Chinese parents. There was no family history of note. He was thriving and developing normally until 10 months of age. He developed high swinging fever secondary to an episode of urinary tract infection. Physical examination during that illness revealed an isolated non tender hepatomegaly with a liver span of 8 cm span at the mid-clavicular line. There was no jaundice, lymphadenopathy or splenonomegaly. After the urinary tract infection settled down and on subsequent follow up, the hepatomegaly persisted. A complete blood count revealed normal hemoglobin, white cell and platelet counts. Liver function tests showed normal bilirubin and albumin levels but elevated transaminase (Table 1).

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Coagulation profile, creatine phosphokinase, urate, non-fasting cholesterol, triglycerides and alpha feto protein were normal. Ultrasound examination revealed an enlarged liver with normal echo texture. No intrahepatic or extrahepatic biliary ductal dilatation was identified. The gallbladder and spleen were normal. Computerized tomography of the abdomen showed a homogenous diffusely enlarged liver with no calcification or focal lesions. An infection screen was also performed. The results were negative for hepatitis A IgM, hepatitis B surface antigen and total core antibody, hepatitis C PCR and hepatitis C antibody as well as negative CMV PCR, EBV PCR, and HIV antibody.

Metabolic workup revealed a normal serum copper and ceruloplasmin level. Alpha 1 antitrypsin PI type was MM. Serum amino acids and urine organic acids were both normal. An autoimmune panel revealed a positive C-ANCA antibody with a titre of 1:160. However anti-liver/kidney microsome, anti-smooth muscle antibody and antinuclear antibody were all negative.

Despite the persistent hepatomegaly, the patient remained well clinically with satisfactory growth and development. The levels of ALT and AST fluctuated and increased at times to over 1000 U/L. Serum bilirubin, albumin and coagulation profile remained normal. He underwent a laparoscopic wedge liver biopsy at 19 months of age. The biopsy revealed expanded hepatocytes with both lipid and glycogen elements within the cytoplasm. No extensive fibrosis or evidence of inflammation was present. Electron microscopy of the hepatocytes did not reveal any characteristic abnormality in the mitochondrial appearance or number. There were no characteristic features of the lipid and glycogen inclusions in the hepatocytes. Overall, the liver biopsy findings were suggestive of either a glycogen storage disease or a cholesterol ester storage disease. A subsequent acid lipase level was checked to be normal. The biochemical assays of enzymes in the phosphorylase system are summarized in Table 1. There was virtually absence of residual phosphorylase b kinase (<2% of reference median).

Patient 2 was a 10 year-old boy who first presented at 18 months of age with gross hepatomegaly found incidentally during a gastroenteritis illness. He was the third child in his family with two healthy elder sisters. Parents were non consanguineous ethnic Chinese. There was no family history of note. He was born at term after an uneventful pregnancy with a birth weight of 3 kg. Other than mild neonatal jaundice, there were no other perinatal problems. There was no prolonged jaundice, seizures or documented hypoglycemia. Borderline delay in developmental milestone especially in the gross motor aspect was noted at initial presentation. At the chronological age of 26 months, he was assessed to have a gross motor developmental age of 13 months by the Peabody developmental scale.

Table 1
Biochemical assays of enzymes in phosphorylase system in 2 patients.

	Patient 1	Patient 2
Plasma		
AST	424 U/L (5–45)[a]	n.a.
ALT	697 U/L (20–60)	100–1300 U/L (<58)
GGT	243 U/L (5–16)	89 U/L (<61)
Leukocyte enzyme assays		
Phosphorylase b kinase	0.8 μmol/min/gHb (10–90)	5 μmol/min/gHb (10–90)
Glycogen	206 μg/gHb (10–120)	n.a.
Glycogen debrancher enzyme		123 nmol/h/mg (20–97)
Phosphorylase a (−AMP)		1.3 (0.3–3.7)
Phosphorylase a (+AMP)		5.2 (2.4–10.4)
Phosphorylase a/Total	0.13 (0.42–0.78)	0.25 (0.42–0.78)

[a] Reference ranges are given in ().

Initial investigations revealed a normal blood count and clotting profile. Liver function tests showed elevated transaminase (Table 1). Bilirubin, alkaline phosphatase and albumin levels were all normal. Random glucose, urate, ammonia, lactate, lipid profile, ferritin, alpha-1-antitrypsin, copper, cerculoplasmin, alpha feto protein and creatine phosphokinase levels were also normal. Viral hepatitis serology screen including Epstein-barr virus, hepatitis A, B and C, cytomegalovirus, toxoplasmosis and human immunodeficiency virus were all negative. Immunological markers ANA and anti-smooth muscle antibody were also negative.

An abdominal ultrasound revealed hepatomegaly with diffuse increase in liver edrogenecity. The biliary tree was normal. Both the spleen and kidneys were not enlarged. Computerized tomography of the abdomen revealed an enlarged hyperdense liver with no focal lesion.

Patient 2 underwent an ultrasound guided liver biopsy at 2 years of age. The biopsy showed fatty change with congestion in the hepatocytes. This finding was thought to be consistent with some form of glycogen storage disease. In the absence of hypoglycemia and a stable clinical condition, glycogen storage diseases type III, VI or IX were thought to be clinically compatible. Peripheral blood was assayed for the phosphorylase system and summarized in Table 1. Significant residual enzyme activity was present in RBC (5 μmol/min/gHb, 10% of reference median).

Since confirmation of the diagnosis at 3 years of age, patient 2 had remained well with normal growth and development. There was none documented nor symptoms suggestive of hypoglycemia. He attended normal school with average academic performance. The previous mild gross motor delay resolved with no demonstrable hypotonia or muscle weakness on follow up. The degree of hepatomegaly did not progress with time. His liver span of 11–12 cm had remained the same over the last few years.

3. Methods

Genomic DNA was extracted from peripheral leukocytes using commercial DNA extraction kits. All PCRs were performed as described before [8] with some modification. The PCR was set up in a total volume of 25 μl containing 5 μl of 5× Phusion HF buffer, 0.2 μl of Phusion™ Hot Start High Fidelity DNA Polymerase (FINNZYMES), 0.4 μl of 10 mM dNTPs, 0.5 μl of 10 μM each forward and reverse primers, 25 ng of DNA template and 6 μl of 5× concentrated Combinatorial Enhancer Solution. The detection of mutation was carried by PCR (98 °C 2 min; (98 °C 1 min, 55 °C 1 min, 72 °C 2 kb/1 min) ×35 cycles; 72 °C 10 min) and was performed on The Veriti® 96-Well Thermal Cycler (Applied Biosysem). Afterwards, PCR products were sequenced in both directions. The results were analyzed by Seqman module in DNASTAR Lasergene 7.0 (DNASTAR) and BioEdit Sequence Aligment Editor Version 7.0.5.3. Computer based-calling errors were corrected by further inspection of chromatograms manually. In order to determine the allelic frequencies in the population, 150 samples (300 chromosomes) were screened using restriction fragment length polymorphism (RFLP). Briefly, the mismatch primers for PHKA2 mutations were designed (Supplementary table) and PCR (96 °C 12 min; (96 °C 1 min, 63 °C 45 s, 72 °C 45 s) ×35 cycles; 72 °C 5 min) was performed followed by Fau I or Pst I restriction digestion at 37 °C overnight. The results were analyzed using agarose gel electrophoresis.

4. Result and Discussion

The high variability in clinical phenotype of glycogen storage disease type IX often hinders the patients from accurate diagnosis. Although the measurement of phosphorylase kinase activity in blood cells is widely used for diagnosis, in some cases, significant residual activity has been reported in some patients. Therefore, we used DNA