Pediatrics and Neonatology (2019) 60, 463–466

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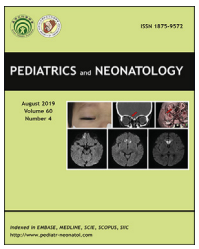

Short Communication

Clinical and genetic characteristics of three Chinese patients with glycogen storage disease type IXα

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Received Apr 23, 2018; received in revised form Mar 24, 2019; accepted May 16, 2019
Available online 25 May 2019

1. Introduction

Glycogen storage disease (GSD) IX is caused by a deficiency of phosphorylase kinase (PHK, EC.2.7.1.38), which plays a critical role in regulating the release of glucose from glycogen, resulting in the storage of glycogen.[1] PHK is composed of four subunits, namely, α, β, γ and δ. Each of the subunits is tissue-specific and encoded by different genes. Subunit α has two isoforms, GSD IXα1 and IXα2 (MIM #306000), both of which are encoded by the *PHKA2* gene (MIM *300798), these isoforms are involved in liver glycogenosis. The clinical phenotypes of patients with both GSD IXα isoforms are variable and include hepatomegaly, short stature, liver dysfunction, hypoglycemia, hyperuricemia, hyperlipidemia, fasting ketosis, and mild motor delay.[2] These clinical and biochemical manifestations improve and even disappear with age. Recent studies have also shown that some untreated children may develop growth delay and psychological distress, and adult patients have an elevated risk of bone fracture.[3,4] Thus, to make a definitive and timely diagnosis of the GSD types or subtypes mainly depends on molecular genetic studies of the relevant genes.

The *PHKA2* gene is located at Xp22.2–22.1 and contains 33 exons.[5] To date, the Human Gene Mutation Database (HGMD) has reported over 110 mutations in the *PHKA2* gene associated with GSD IXα, including missense, nonsense, splicing, small deletions, small insertions and gross deletions variants. GSD IXα1 results from the truncation or disruption of the PHKA2 protein, while GSD IXα2 results from missense mutations or small in-frame deletions and insertions in the *PHKA2* gene.[6] This suggests that the biochemical differences between the two isoforms of GSD IXα are due to the different nature of the disease-causing mutations in the *PHKA2* gene. However, the enzyme activity of PHK is difficult to verify in vitro. Thus far, 21 cases of GSD IXα have been detected in Chinese patients with mutations of the *PHKA2* gene in recent years.[7–10] However, more cases are needed to analyze the relationship between the genotypes and phenotypes of GSD IXα. Here, the clinical manifestations, laboratory findings (liver biopsy) and *PHKA2* gene mutations in three patients with unrelated Chinese parents were reported.

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https://doi.org/10.1016/j.pedneo.2019.05.007

2. Case reports

Three male patients had the main clinical manifestation of liver dysfunction and were suspected of having inherited metabolic liver disease. Informed consent was obtained from their parents. A summary of the clinical and abnormal laboratory characteristics of the three patients is shown in Table 1. All three patients are male, and at the clinical onset of disease, all patients were younger than two years old (24 months). Abdominal distension with hepatomegaly and increased serum transaminase levels were the major clinical manifestations, although the disease severity was variable. Some biochemical features of the three patients remained abnormal after aging and treatment. Their aminotransferase, aspartate aminotransferase, triglyceride and lactate dehydrogenase values were clearly higher than the normal ranges. The levels of r-glutamyl transferase, total bile acid, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and lactate varied among the three patients. The immunoglobulin E (IgE) level was higher than the normal range in all three patients. After symptomatic treatment, the hypoglycemia in patients 1 and 2 was relieved. Serum markers for viral hepatitis, Epstein–Barr virus and cytomegalovirus were all negative. None of the common

Table 1 Summary of the clinical and abnormal laboratory characteristics of the three patients with GSD IXα caused by mutations in *PHKA2*.

Patient	1	2	3
Sex	Male	Male	Male
Age at onset (months)	16	24	24
Age at diagnosis (months)	22	48	30
Height at diagnosis (cm)	78 (3%)	82 (10%)	86 (10%)
Weight at diagnosis (kg)	10.5 (25%)	14 (25%)	11.7 (10%)
Major clinical manifestations	Increased transaminase, Hepatomegaly, Hypoglycemic	Abdominal distension, Increased transaminase, Hepatomegaly, Growth retardation	Abdominal distension, Increased transaminase, Hepatomegaly
ALT (9–50 U/L)[a]	98	268	312
AST (5–60 U/L)[b]	151	173	346
r-GT (10–60 U/L)[c]	75	47	111
TBIL (2–17 μmol/L)[d]	8.2	5.5	5.3
TBA (0–13 μmol/L)[e]	7.3	7.2	35
CK (45–390 U/L)[f]	44	134	86
TG (0.23–1.7 mmol/L)[g]	3.72	2.85	2.21
TC (3.4–5.2 mmol/L)[h]	4.38	5.65	4.86
HDL-C (0.88–1.8 mmol/L)[i]	0.59	0.98	1.15
LDL-C (<3.37 mmol/L)[j]	3.86	3.76	3.31
Glu at onset (3.5–5.7 mmol/L)[k]	3.01	3.15	–
Glu at diagnosis (3.5–5.7 mmol/L)[k]	3.6	3.85	5.2
LDH (159–322 U/L)[l]	546	390	359
LAC (0.9–1.7 mmol/L)[m]	4	1.7	3.3
IgG (5.0–10.6 g/L)[n]	8.07	9.57	6.96
IgA (0.34–1.38 g/L)	0.76	1.47	1.09
IgM (0.44–1.44 g/L)	1.48	1.19	1.26
IgE (0–60 IU/ML)	186	1600	201
Liver biopsy	NR[o]	PAS(+), Liver cells mass increased, large number of glycogen particles	NR
PHKA2 gene mutations	c.3614C > T p.Pro1205Leu	c.2810_2811delAA p.Glu937fs	c.3334G > A p.Glu1112Lys
Other	Mother had same homozygous mutation, increased transaminase were found at about 6 years old (pneumonia in hospital)	–	Elder brother had the same clinical feature

Note: All laboratory values were obtained from the testing performed prior to treatment for GSD in our hospital. a: ALT, alanine aminotransferase; b: AST, aspartate aminotransferase; c: r-GT, r-glutamyl transferase; d: TBIL, total bilirubin; e: TBA, total bile acid; f: CK, creatine kinase; g: TG, triglyceride; h: TC, total cholesterol; i: HDL-C, high-density lipoprotein cholesterol; j: LDL-C, low-density lipoprotein cholesterol; k: Glu, glucose; l: LDH, lactate dehydrogenase; m: LAC, lactate; n: Ig, immunoglobulin; o: NR, not recorded.

inherited metabolic diseases were found through spectrum analysis of serum amino acids, acyl-carnitine and urine in the three patients. Abdominal ultrasound revealed marked hepatomegaly in all patients.

To identify the gene mutations in these patients, Next-Generation Sequencing including exome sequencing with a panel was performed, the panel included more than 300 genes associated with hepatopathy. In patient 1, we identified a previously described hemizygous c.3614C > T (p.Pro1205Leu) missense mutation in exon 33 of the *PHKA2* gene, and his mother has a homozygous mutation of this locus.[11] A *de novo* hemizygous c.2810_2811delAA (p.Glu937Glyfs*4, exon 26) mutation of the *PHKA2* gene was found in patient 2. In patient 3 and his elder brother, a novel missense mutation in exon 31 of the *PHKA2* gene, c.3334G > A (p.Glu1112Lys), was found, which was inherited from his mother. The pathogenicity of this missense mutation was predicted by SIFT and PolyPhen-2 software, and the results were damaging (0) and probably damaging (0.973), respectively. The presence of these mutations was confirmed by Sanger sequencing (Fig. 1).

Only patient 2 who was suspected of having a metabolic abnormality underwent a liver biopsy after his parents provided informed consent. The biopsy revealed hepatocytes swelling with abundant glycogen elements within the cytoplasm. PAS staining for glycogen was positive. No extensive fibrosis or evidence of inflammation was present (Supplementary Fig. 1).

The follow-up for all three patients and the elder brother of patient 3 revealed that they all consume raw corn starch before meals, in amounts depending on their weight. The abnormal values of the biochemical tests are close to the normal ranges now. Their growth is similar to that of their peers. The medical history of patient 1's mother showed that she was admitted to the hospital for pneumonia when she was approximately 6 years old, and a biochemical examination revealed high transaminase levels. The parents of patient 1's mother are consanguineous, which resulted in a homozygous mutation of the *PHKA2* gene in the mother. At present, the grandparents do not have any clinical phenotypes indicative of GSD disease, and their genotypes are unknown. This shows that the c.3614C > T mutation of the *PHKA2* gene is pathogenic and that the clinical and biochemical manifestations could disappear with increasing age.

3. Discussion

GSD IX is an important type of GSD, and its biochemical and genetic diagnoses are complicated because of the phenotypic and genetic heterogeneity, with the genotypes and phenotypes overlapping with those of other GSDs.[12] Therefore, an early and accurate diagnosis of patients with the GSD IXα isoform is necessary. In this article, we identified one previously described and two novel *PHKA2* gene variants in three patients suspected of having GSD. Combined with the results of the clinical, biochemical and molecular genetic testing, one patient with a frameshift mutation was diagnosed with GSD IXα1, and two patients with missense mutations were diagnosed with GSD IXα2.

Consistent with the results of previous reports, the first onset age was younger than two years in these three patients.[8] Our results reconfirmed that hepatomegaly and increased serum transaminase levels are the most common characteristics of patients with GSD IXα. In addition, the biochemical analysis showed that the value of IgE was significantly higher than the normal range in all three patients. In our experience, some other types of GSD also result in elevated IgE levels, but no published studies have reported that relationship thus far. However, some reports show that elevated serum IgE levels can be detected in patients with acute/chronic liver inflammation (viral infection or autoimmune inflammation).[13–15] Whether the elevated serum IgE level is related to the degree of liver inflammation, severity of the disease, or prognosis remains unknown. The limited number of cases in this paper is not sufficient to determine these associations. The significance of increased IgE levels in children with GSD should be further studied and summarized. Therefore, the results of these biochemical examinations can only indicate that the children are suspected of having liver disease but cannot be the basis of a definitive diagnosis. Thus, timely genetic testing is essential.

In this article, two missense mutations and a deletion mutation were detected. We did not identify any mutations previously reported in Chinese patients in these three patients. The missense mutation c.3614C > T (p.Pro1205Leu) was first described in a Dutch boy in 1995.[11] The novel mutation c.2810_2811delAA (p.Glu937Glyfs*4) was a *de novo* deletion mutation, and c.3334G > A (p.Glu1112Lys) was a novel missense mutation inherited from the patient's

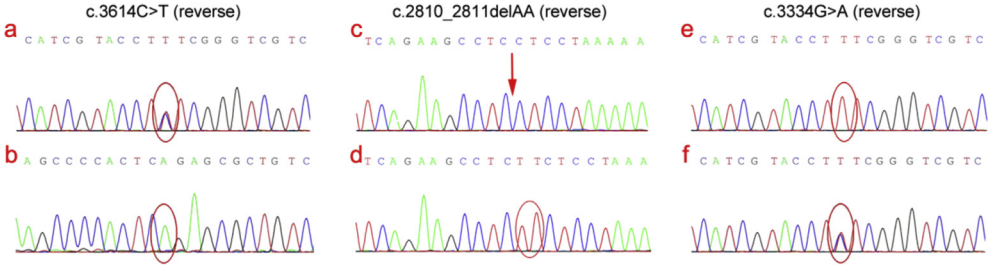

Figure 1 Sanger sequencing result of *PHKA2* gene. Patient 1 (a) was found to have a hemizygotic mutation (c.3614C > A), which was inherited from his mother (b), who was found to have a homozygous mutation. Patient 2 (c) was found to have a novel c.2810_2811delAA hemizygotic mutation. The mother of patient 2 (d) had no mutations of the *PHKA2* gene. Patient 3 (e) was found to have a maternally inherited hemizygotic c.3334G > A mutation. His mother (f) was found to have a heterozygous mutation. The red circles indicate the nucleotide mutation sites, and the red arrow indicates the nucleotide deletion position.

mother. The c.2810_2811delAA mutation resulted in a truncation of the PHKA2 protein, containing 939 amino acids. The predicted pathogenicity of c.3334G > A was likely pathogenic, as assessed with SIFT and PolyPhen-2 software. Hendrickx et al. identified *PHKA2* gene mutations in 14 patients with GSD IXα, 1 and 2.[6,16] Based on these analyses, patients 1 and 3, who had missense mutations, were diagnosed with GSD IXα2, and patient 2, who had a small deletion resulting in the truncation of the PHKA2 protein, was diagnosed with GSD IXα1. In addition to the increased transaminase level, hepatomegaly and hypoglycemia, growth retardation was also noted in patient 2 (Table 1). These results indicate that the clinical phenotype of GSD IXα1 may be more severe than that of GSD IXα2. However, there is no definite conclusion about the relationship between genotype and phenotype. More cases of GSD IXα need to be reported.

A definitive diagnosis can be achieved by performing a liver biopsy to assess the nature and severity of the liver disease.[17] Considering the risks associated with liver biopsy, only patient 2 underwent a liver biopsy after his parent provided informed consent. The liver biopsy demonstrated that the hepatocytes appeared swollen with abundant glycogen elements within the cytoplasm, suggesting GSD. This data, combined with the molecular genetic testing results of patient 2, allowed the patient to be accurately diagnosed with GSD IXα1. Thus, when genetic testing reveals mutations in the *PHKA2* gene, liver biopsy can be performed to determine the occurrence and severity of GSD, which is helpful for obtaining a clinically accurate diagnosis.

In conclusion, two novel mutations detected in this study can further enrich the mutation database of the *PHKA2* gene in the Chinese population and worldwide. This report of three cases helps clarify the relationships between the phenotypes and genotypes of GSD IXα patients. The application of molecular genetic testing can provide an early and accurate diagnosis of patients suspected of having GSD.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pedneo.2019.05.007.