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Variability of disease spectrum in children with liver phosphorylase kinase deficiency caused by mutations in the *PHKG2* gene

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Abstract

Liver phosphorylase b kinase (PhK) deficiency (glycogen storage disease type IX), one of the most common causes of glycogen storage disease, is caused by mutations in the *PHKA2*, *PHKB*, and *PHKG2* genes. Presenting symptoms include hepatomegaly, ketotic hypoglycemia, and growth delay. Clinical severity varies widely. Autosomal recessive mutations in the *PHKG2* gene, which cause about 10–15% of cases, have been associated with severe symptoms including increased risk of liver cirrhosis in childhood. We have summarized the molecular, biochemical, and clinical findings in five patients, age 5–16 years, diagnosed with liver PhK deficiency caused by *PHKG2* gene mutations. We have identified five novel and two previously reported mutations in the *PHKG2* gene in these five patients. Clinical severity was variable among these patients. Histopathological studies were performed for four of the patients on liver biopsy samples, all of which showed signs of fibrosis but not cirrhosis. One of the patients (aged 9 years) developed a liver adenoma which later resolved. All patients are currently doing well. Their clinical symptoms have improved with age and treatment. These cases add to the current knowledge of clinical

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variability in patients with *PHKG2* mutations. Long term studies, involving follow up of these patients into adulthood, are needed.

Keywords

Phosphorylase b kinase deficiency; glycogen storage disease type IX; *PHKG2* gene; liver adenoma; hypoglycemia

1. Introduction

Deficiency of liver phosphorylase b kinase (PhK), also known as glycogen storage disease type IX (GSD IX), is one of the most common forms of glycogen storage disease. It accounts for about 25% of cases and has an estimated frequency of 1 in 100,000 [1]. PhK is a key regulatory enzyme in glycogen breakdown and metabolism. In response to physiological conditions, PhK activates glycogen phosphorylase which catalyzes the sequential cleavage of glucosyl units from glycogen to release glucose 1-phosphate [2, 3]. PhK is a heterotetramer that is composed of four copies each of alpha, beta, gamma, and delta (calmodulin) subunits [4]. The gamma subunit contains the catalytic site. Its activity is regulated by the phosphorylation state of the alpha and beta subunits, as well as by calmodulin via calcium levels [4]. Different isoforms of PhK exist due to tissue-specific expression and alternative splicing of different subunit genes. In liver, the alpha, beta, and gamma subunits are encoded by the *PHKA2* gene (OMIM# 300798; Xp22.2-p22.1), the *PHKB* gene (OMIM# 172490; 16q12-q13), and *PHKG2* gene (OMIM# 172471; 16p12.1-p11.2) respectively. However, recent studies have suggested that these genes are more widely expressed than previously thought [5]. To date, mutations in each of these genes have been identified in individuals with liver PhK deficiency [1, 6–10].

Children with liver PhK deficiency typically present in the first two years of life with hepatomegaly, growth retardation, and elevated serum transaminases and plasma triglycerides. Ketotic hypoglycemia and hypotonia may also be present. Available literature suggests that the clinical course is usually mild when compared to other common liver GSDs, and that symptoms typically improve with age [3, 6, 11, 12]. Liver cirrhosis and adenomas, which are common in some other types of liver GSD, have been reported less frequently in individuals with liver PhK deficiency [13]. Over recent years, however, a wide variation in clinical severity among patients with liver PhK deficiency has been recognized and some general correlations between the gene defect and clinical severity have been made [7, 8, 14, 15]. Mutations in the X-linked *PHKA2* gene are the most common cause of liver PhK deficiency, accounting for about 75% of cases [7, 8]. Although historically reported to have a relatively benign course, a wide spectrum of clinical severity resulting from mutations in *PHKA2* has emerged recently [7, 14–18]. Mutations in the *PHKB* gene cause an autosomal recessive form of liver PhK deficiency that, so far, has been associated with mild symptoms [7, 8, 10, 19, 20]. By contrast, mutations in the *PHKG2* gene often cause more pronounced biochemical and clinical abnormalities including an increased risk for developing liver cirrhosis in childhood [1, 7, 8, 21–24]. Here, we report the clinical symptoms, laboratory findings, and *PHKG2* gene changes in five individuals with liver PhK deficiency in order to further delineate the variability in clinical phenotype caused by mutations in this gene.

2. Materials and methods

2.1 Patients

The patients were recruited under a research protocol in accordance with the Institutional Review Board requirements of Duke University or the University of Florida Health Center. *PHKG2* gene sequence analysis was performed by the Duke Molecular Diagnostics Laboratory (patients 1, 2 and 5) or in another clinical diagnostic laboratory (patients 3 and 4). Medical records, including clinic notes and reports from clinical laboratory testing, were reviewed. Laboratory data obtained included blood glucose and ketones, serum transaminases, triglyceride, cholesterol, creatine kinase (CK), bilirubin, and partial thromboplastin time (PTT) from standard clinical laboratory testing. Each patient was evaluated by a metabolic dietician, and data on treatment with cornstarch and protein supplementation was collected.

2.2 PhK enzyme activity

PhK enzyme activity were measured in erythrocytes and/or frozen liver biopsy samples in the Glycogen Storage Disease Laboratory at Duke University Medical Center using standard spectrophotometric methods[25]. The residual enzyme activity was measured indirectly by measuring the amount of glucose or phosphate released using glucose reagent (Infinity™; Cat No. TR15421) or phosphate reagent (Inorganic Phosphorous; Cat No. TR30026) from ThermoScientific (Fisher Diagnostics, Middletown, VA, USA). Enzyme activity was expressed as $\mu\text{mol}/\text{min}/\text{g}$ hemoglobin (erythrocytes) or $\mu\text{mol}/\text{min}/\text{g}$ tissue (liver).

2.3 Molecular analysis

All nine coding exons and flanking intronic sequences of the *PHKG2* gene (NM_000294) were amplified by polymerase chain reaction (PCR) using genomic DNA (extracted with Qiagen Puregene Blood Core Kit B) using standard PCR and sequencing techniques. Amplicons were purified using Qiagen QIAquick® PCR Purification kit and were bidirectionally sequenced using ABI3730 DNA Analyzer through the Duke University Analysis facility. Sequence changes were named according to the recommendations of the Nomenclature Working Group [26, 27] and the Human Genome Variation Society (www.hgvs.org). The effect of sequence changes identified at exon/intron boundaries was predicted using the Berkeley Drosophila Genome Project (BDGP) Splice Site Prediction Tool (http://www.fruitfly.org/seq_tools/splice.html). To determine if any novel missense mutations identified in this study are common variants we searched the NCBI SNP database (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=Snp>). The pathogenicity of novel missense mutations was predicted using the PolyPhen-2 algorithm [28]. A summary of DNA testing performed for each patient is shown in Table 2.

3. Results

3.1 Clinical history

A summary of the clinical and laboratory features for the five patients included in this study is shown in Table 1. All patients in our case series had hepatomegaly, elevated serum transaminases, and fasting hypoglycemia, although disease severity was variable. Ketosis was observed in some cases. Over time, with age and treatment, there was general improvement in the biochemical abnormalities for all of the patients. In four of the patients, hepatomegaly has lessened, or even normalized (Patient 2). Growth has improved for all of the patients, except Patient 5 who is now 5 years old and continues to follow a growth curve below the 5th percentile. Based on parental heights, his estimated final height would be at the 25th percentile.

3.2 Treatment

Treatment regimens varied for the 5 patients in this study. Treatment involved multiple doses of uncooked cornstarch (CS) daily, varying from 0.5g–1.8 g CS/kg body weight. Some patients received higher doses (e.g. 2g CS/kg body weight) of cornstarch at bed time in order to maintain glucose levels through the night time. Cornstarch doses were based on individual patient needs. Patients 1, 3, 4, and 5 also received protein supplementation, providing a total protein intake of 3–4g/kg daily. Over time, the need for cornstarch has decreased for Patients 1 (13 years old) and 2 (16 years old). Both of these patients now take only one dose of cornstarch, at night time (1.8g CS/kg plus 0.4g protein supplement/kg, and 0.3g CS/kg respectively). The cornstarch and protein doses for Patient 4 were recently increased after she was experiencing nausea and low stamina. Patients 2 and 5 used a G-tube for feedings, and patient 5 still continues to use a G-tube for night-time treatment.

3.2 *PHKG2* sequence analysis

Overall, five novel mutations (c.647+5G>T, p.Glu8*, p.Gln83*, p.Lys53del, and p.Tyr358Cys) and two previously reported mutations (c.96-11G>A and p.Trp300*) [24, 29] were identified in the five patients (Table 2). The finding of five novel mutations in this series of patients brings the total number of mutations in *PHKG2*, reported in full-length papers, to 25 (8 missense, 8 nonsense, 4 frameshift, 4 splice site, and one small in frame deletion) (Supplementary Table 1). In addition, a known polymorphism in *PHKG2*, c.271+38C>T (rs4889504), was found in homozygosity in patients 1 and 3.

4. Discussion

Mutations in *PHKG2* have been associated with more severe clinical and biochemical abnormalities. These may include extremely low PhK activity in liver, pronounced tendencies to hypoglycemia, abnormal glucagon response, and increased risk of liver cirrhosis [1, 7, 8, 21–24, 30]. To date, 17 individuals (16 probands and one sibling) with PhK deficiency, caused by *PHKG2* mutations, have been reported in full length papers in the medical literature (Supplementary Table 1). Strikingly, 13 patients for whom liver pathology was reported had liver fibrosis. In seven of those cases there was also a progression to liver cirrhosis, often in childhood, and one of the patients developed adenomas [22]. In contrast to the published reports of patients with *PHKG2* mutations and progression to liver cirrhosis early in life, none of our patients (ages 5–16 years) have developed cirrhosis thus far. However, all our patients for whom histology has been performed have fibrosis (n=4). It is not possible to predict whether any of these patients may develop cirrhosis later in life, similar to other patients who developed cirrhosis in adulthood [8]. One of our patients also developed an adenoma at age 9 years of age although it later resolved. To our knowledge, adenoma has only been reported in one other patient with confirmed liver PhK deficiency, and that patient also had mutations in *PHKG2* [22]. Further studies are needed to determine why some patients with PhK deficiency seem to be at increased risk to develop liver cirrhosis and adenoma compared to others. Other causes of liver cirrhosis, such as hepatitis A, hepatitis B, hemochromatosis, and alpha1-antitrypsin deficiency should also be ruled out in these cases.

Treatment varied for all 5 patients in our study. Beyond frequent feedings, there is no consensus regarding prevention of hypoglycemia. Raw cornstarch therapy, with dosing anywhere from 0.3 to 2.5 g/kg body weight every 6 hours or just at bedtime for the overnight fast, is often used but may not be necessary for all patients. High protein diet and/or supplementation is also helpful [6, 15, 18, 24, 31]. In severe cases, continuous night-time tube feedings in addition to daytime cornstarch may be needed [15]. Monitoring of ketones, the presence of which indicates mobilization of fats and may signal pending or concurrent

hypoglycemia, has been suggested [6]. However, further studies are needed to determine the exact relationship between ketone levels, metabolic control, hypoglycemia and appropriate dietary management of these patients.

Mild muscle weakness was found in Patient 1 and Patient 4 in early childhood (now resolved) without elevation of serum creatine kinase. As reported in other cases, this may be a secondary phenomenon, perhaps related to altered energy metabolism affecting the muscle [8, 22]. However, recent in silico studies have shown presence of expressed sequence tags (ESTs) from both *PHKA2* and *PHKG2* in muscle as well [5] suggesting that these genes may, perhaps, have some function in muscle. Regardless of the cause, muscle weakness in patients with hepatic PhK deficiency warrants close follow up to ensure that energy needs are being met and that another cause is not missed.

Interestingly, abnormalities in urine organic acids were found in the patients in whom this was analyzed, with elevation of 3-methylglutaconic acid being the most consistent abnormality (Patients 1 and 5). Of note, elevated 3-methylglutaconic acid has previously been reported to be elevated in patients with X-linked GSD IX caused by mutations in the *PHKA2* gene, and also in patients with GSD I and III [15, 32]. The underlying cause of elevation in 3-methylglutaconic acid is unknown but has been suspected to reflect abnormal cholesterol metabolism or general mitochondrial dysfunction [32]. Of note, in our two patients, cholesterol level was normal or only mildly elevated at the time of urine organic acids analysis.

We identified five novel mutations (c.647+5G>T, p.Glu8*, p.Gln83*, p.Lys53del, and p.Tyr358Cys) and two previously reported mutations (c.96-11G>A and p.Trp300*) [24, 29] in our five patients (Table 2). All of these changes are predicted to affect the normal function of the liver/testis gamma subunit of PhK, which is encoded by *PHKG2*. The nonsense mutations (p.Glu8*, p.Gln83*, and p.Trp300*) are expected to be pathogenic due to nonsense mediated decay of mRNA transcripts with premature stop codons [33]. Based on the 3D structure of *PHKG2*, and comparison with other protein kinases, two of the other mutations, p.Lys53del and c.96-11G>A, are predicted to affect ATP binding. Lys53 is a highly conserved residue. It is part of the VIAK motif and is required for ATP binding and for stabilizing the active state of the protein. The salt bridge between the lysine residue in the VIAK motif with a conserved residue in helix alpha-C is a hallmark of the active kinase state. Deletion of Lys53 is, therefore, predicted to result in inactivation of the protein kinase function of *PHKG2* (<http://www.rcsb.org/pdb/explore/literature.do?structureId=2Y7J>; Stephen Knapp, personal communication; [34]). The insertion of SerSerCys between Gly31 and Arg32, due to the predicted creation of a new splice site by c.96-11G>A, is also expected to be deleterious. This insertion is located in the phosphate-binding loop (P-loop), a highly conserved, glycine-rich motif that also plays an essential role in ATP binding (<http://www.rcsb.org/pdb/explore/literature.do?structureId=2Y7J>; Stephen Knapp, personal communication; [34]). Tyr358 is outside the catalytic domain. The effect of substitution of this amino acid for Cys is less clear but is predicted to be deleterious by the PolyPhen-2 algorithm [28].

The multiple genes involved in PhK deficiency and the large size of the *PHKA2* and *PHKB* genes complicates genetic testing for PhK deficiency. A recent study involving sequence analysis of *PHKA2*, *PHKB* and *PHKG2*, in 34 individuals with known or suspected PhK deficiency, found that 3 of the 4 most severely affected individuals, with low fasting tolerance, hypoglycemia, and severe PhK deficiency, had mutations in *PHKG2*. No mutations in any of these genes were found in the 4th patient. This data, and prior reports, suggests that sequencing of the *PHKG2* gene should be a priority in individuals with severe symptoms. However, wide clinical severity has been recognized in patients, predominantly

males, with mutations in *PHKA2*. Due to presence of liver cirrhosis and recurrent hypoglycemia in some patients, there can be significant overlap between the clinical features reported in patients with *PHKG2* mutations and those with mutations in *PHKA2* [15, 17, 18]. Conversely, milder manifestations have been found in some individuals with mutations in *PHKG2* [1]. Patients with mutations in *PHKB* also present similarly to some patient with a mutations in *PHKA2* [8]. Gender and family history can be taken into account when prioritizing these genes for sequencing [6]. The advent of gene panels, and whole exome sequencing, can also facilitate genetic testing for these patients, particularly for those who have not had a liver biopsy and could have other GSD subtypes in the differential, such as GSD type VI (liver glycogen phosphorylase deficiency) or GSD type III (glycogen debranching enzyme deficiency) [30, 35].

In summary, we report five new patients with *PHKG2* mutations. All patients have hepatomegaly and have required dietary intervention to maintain normal blood glucose levels, although severity of hypoglycemia, and other features including growth delay, varied among the patients. Patient 2 also has short chain acyl-CoA dehydrogenase (SCAD) deficiency, and it is not known whether, in the context of glycogen storage disease, this diagnosis may have a synergistic effect [36]. In keeping with previous reports of patients with *PHKG2* mutations, fibrosis was found in all four patients for whom liver histology was available and one patient developed an adenoma at age 9 years. However, none of the patients has developed liver cirrhosis yet. Detailed long-term natural history studies are required to determine whether these patients may be at increased risk to develop additional complications, such as liver cirrhosis or hepatocellular carcinoma, later in life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

We report the clinical and laboratory features for 5 patients with *PHKG2* mutations.

A literature review of reported patients with *PHKG2* mutations is presented.

We identified five novel and two previously reported mutations in the *PHKG2* gene.

There is variable clinical severity of symptoms associated with *PHKG2* mutations.

Table 1

Summary of clinical and laboratory features of 5 patients with liver PhK deficiency caused by mutations in *PHKG2*.

Patient	1	2	3	4	5
Presentation	Hypoglycemic seizures at age 2 years, hepatomegaly	Hypoglycemic seizures ending in respiratory arrest at age 12 months, hepatomegaly, prominent cheeks, hypotonia	Hepatomegaly (16 cm below RCM) noted on physical exam at 11 months	Hepatomegaly noted on physical exam at 4 months; elevated serum transaminases	Decrease in growth velocity at 9 months old, hepatomegaly (8cm below RCM) noted on physical exam
Age at diagnosis	44 months	12 months	16 months	2 years	12 months
Current age	12 years	16 years	6 years	9 years	5 years
Gender/Ethnicity	Male Caucasian (Hispanic)	Male Caucasian (non-Hispanic)	Female Caucasian (non-Hispanic)	Female Caucasian (non-Hispanic)	Male Caucasian (non-Hispanic)
Hepatomegaly	Yes	Yes	Yes	Yes	Yes
Liver pathology	Bridging portal fibrosis, no steatosis (at 3 years of age)	Mild portal fibrosis, no steatosis (at 12 months of age)	Bridging fibrosis; area of regenerative process but no cirrhosis (at 11 months of age)	Bridging fibrosis (at about 6 months of age); adenoma, 1.7cm diameter, in right lobe of liver (at 9 years of age), resolved	Not done
Fasting hypoglycemia	Yes	Yes	Yes (borderline)	Yes	Yes
Fasting ketosis	Yes	Yes	Yes	Yes	Not detected
AST*	Elevated (20x)	Elevated (20x)	nr	Elevated (10x)	Elevated (10x)
ALT*	Elevated (10x)	Elevated (10x)	Elevated (30x)	Elevated (10x)	Elevated (10x)
Triglyceride*	Mildly elevated	Mildly elevated	Elevated (2x)	Elevated (2x)	Elevated (3x)
Cholesterol	Normal	Normal	Normal	nr	Mildly elevated
Lactic acid*	Elevated (2x)	Mildly elevated	Elevated (2x)	nr	Elevated (2-5x)
Uric acid*	Elevated (1.5x)	Normal	Normal	nr	Mildly elevated
Growth	Normal, but improved on therapy	Growth delay in childhood (<5 th percentile for height)	Normal	Normal, but improved on therapy	Delayed (5 th percentile for height and head circumference)
Development	Mild delay in walking (21 months old)	Mild gross motor delay	Feeding issues caused by sensory texture aversion	Normal	Mild speech delay, feeding issues caused by sensory texture aversion
Other	Mild muscle weakness	short chain acyl-CoA dehydrogenase deficiency	None	Mild muscle weakness (resolved); muscle aches after strenuous activity	Significant mid-foot pronation, slight hind-foot valgus, slight genu valgus requiring orthoses; left hydronephrosis (resolved)

All laboratory values were obtained from the testing done prior to treatment for GSD (cornstarch and protein supplementation).

* Indicates the number of times the upper limit of the normal range.

nr = not recorded, RCM = right costal margin

Serum creatine kinase, bilirubin, total protein, albumin, and prothrombin time were normal for all patients. Echocardiogram was normal (Patients 1, 3, 4, 5)

Patient 2 also has SCAD deficiency, identified by enzyme assay and DNA analysis.

Table 2

PhK activity and *PHKG2* mutations identified in the 5 patients in this study.

Patient	Liver glycogen (3.3+/-1.7%)	PhK (liver) (%control activity)	PhK (erythrocytes) (% control activity)	Genes analyzed	<i>PHKG2</i> allele 1	<i>PHKG2</i> allele 2
1	10.80%	20%	0%	<i>PHKA2</i> , <i>PHKB</i> , <i>PHKG2</i>	c.647+5G>T ^{a,b}	c.647+5G>T ^b
2	17.50%	5%	ND	<i>PHKA2</i> , <i>PHKB</i> , <i>PHKG2</i>	p.Lys53del (c.158_160delAAGA)	p.Glu8X* ^c (c.22G>T)
3	ND	ND	ND	<i>PYGL</i> , <i>PHKG2</i>	p.Gln83* (c.247C>T) ^d	c.96-11G>A ^{d,e}
4	ND	ND	ND	<i>AGL</i> , <i>PHKG2</i>	p.Trp300* (c.900G>A)	p.Tyr358Cys ^f (c.1073A>G)
5	16%	10%	2%	<i>PHKA2</i> , <i>PHKB</i> , <i>PHKG2</i>	c.96-11G>A ^e	No mutation identified ^g

^aThe father of patient 1 is heterozygous for c.647+5G>T. His mother has not been tested.

^bThis mutation is predicted to affect normal splicing.

^cp.Glu8* is listed in dbSNP (rs375063162) but no population frequency data are available.

^dBased on the results of parental testing, the *PHKG2* mutations identified in Patient 3 are in *trans*.

^eThe BDGP Splice Site Prediction tool predicts that the c.96-11G>A substitution creates a new acceptor splice site, nine nucleotides upstream from the normal intron 2 acceptor splice site. If the new splice site is used, it is predicted that an additional three amino acids, SerSerCys, would be incorporated into the protein between amino acids Gly31 and Arg32, potentially disrupting protein function.

^fThe PolyPhen-2 algorithm predicts that p.Tyr358Cys is probably damaging.

^gNo further analyses, such as multiple ligation-dependent probe amplification (MLPA) analysis to look for a large deletion, or sequencing of the promoter or introns was performed to look for a second mutation for Patient 5.