



Case Report

Profound neonatal lactic acidosis and renal tubulopathy in a patient with glycogen storage disease type IX α 2 secondary to a *de novo* pathogenic variant in *PHKA2*

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ABSTRACT

The phenotype of individuals with glycogen storage disease (GSD) IX appears to be highly variable, even within subtypes. Features include short stature, fasting hypoglycemia with ketosis, hepatomegaly, and transaminitis. GSD IX α 2 is caused by hemizygous pathogenic variants in *PHKA2*, and results in deficiency of the phosphorylase kinase enzyme, particularly in the liver. Like other GSDs, GSD IX α 2 can present with hypoglycemia and post-prandial lactic acidosis, but has never been reported in a newborn, nor with lactic acidosis as the presenting feature. Here we describe the clinical presentation and course of a newborn boy with profound neonatal lactic acidosis and metabolic acidosis, renal tubulopathy, and sensorineural hearing loss (SNHL) diagnosed with GSD IX α 2 through exome sequencing. Review of the literature suggests this case represents an atypical and severe presentation of GSD IX α 2 and proposes expansion of the phenotype to include neonatal lactic acidosis and renal tubulopathy.

1. Introduction

Glycogen storage disease (GSD) IX is caused by deficiency of the phosphorylase kinase (PhK) enzyme, which has a major regulatory role in the breakdown of glycogen [1–3]. The PhK enzyme is comprised of 16 subunits, four of each α , β , γ , and δ subunit. Subunits are encoded by various genes (α : *PHKA1* and *PHKA2*; β : *PHKB*; γ : *PHKG1* and *PHKG2*; δ : *CALM1*, *CALM2*, and *CALM3*), with defects in each subunit giving rise to a variable clinical phenotype [2,4]. PhK deficiency is classified depending on the functional effect on the liver and/or muscle isoform. PhK liver deficiency is more common with clinical features including hypoglycemia, fasting ketosis, early onset hepatomegaly and growth restriction. PhK muscle deficiency is considerably more rare, with features entailing exercise intolerance, muscle cramps, myalgia, progressive muscular weakness and myoglobinuria [4,5].

PHKA2 is located on the X chromosome and encodes a portion of the PhK α subunit. *PHKA2*-associated GSD IX, here-in referred to as GSD

IX α 2, represents the most common form of PhK deficiency and is relatively specific to the liver. Individuals within this subgroup can develop hepatic fibrosis that can progress to cirrhosis [2,3,6,7].

Lactic acidosis is considered a common feature for some GSD subtypes, such as GSDIa [1]; however it is not a frequently reported feature of GSD IX α 2 [4,8–10]. While post-prandial lactic acidosis has been reported individuals with GSD IX α 2 [8,10], profound neonatal hypoglycemia with lactic acidosis has not been reported in patients with GSD IX α 2, and rarely renal tubulopathy has been described.

2. Case presentation

A newborn boy with a history of asymmetric intrauterine growth restriction (IUGR; weight 2nd, length 19th, and head circumference 50th percentile) and high-risk screen for trisomy 21 was born at 39 weeks gestation to a 37 year-old father and 33 year-old mother via vaginal delivery, weighing 2500 g and small for gestational age (SGA).

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Prenatal evaluation included normal chromosomal microarray via amniocentesis and normal fetal echocardiogram. Family history was notable for healthy parents and older sister; otherwise noncontributory.

Within the first hour of life, patient underwent hypoglycemia screening due to SGA and was found to have asymptomatic hypoglycemia (minimum 4 mg/dL, Fig. 1) despite breastfeeding on demand. He was then placed on intravenous (IV) dextrose, with failure to wean as he showed two hypoglycemic episodes during the first 3 days of life (Fig. 1). During the second episode (25 mg/dL), critical labs revealed metabolic lactic acidosis (capillary blood gas pH 7.26, lactate 11 mmol/L, bicarbonate 12 mmol/L, anion gap 17), elevated beta-hydroxybutyrate (0.61 mmol/L) and age-appropriate levels of cortisol, insulin, growth hormone, and transaminases. Patient was managed on IV dextrose at ½ maintenance for the first week of life achieving euglycemia with breastfeeding and formula on demand, but lactic acidosis did not resolve (range 4–6 mmol/L, Fig. 1). State newborn screen was reported as normal. At one week of age, patient was transferred to a level 4 neonatal intensive care unit due to recurrent hypoglycemia and persistent lactic acidosis (Fig. 1).

Biochemical evaluation, including serum carnitine levels, acylcarnitine profile (ACP), plasma amino acids (PAA) and urine organic acids (UOA), were significant for lactic aciduria and mild elevation of *p*-hydroxyphenyllactate in urine. Patient was successfully weaned off IV dextrose on day of life 12 and was recommended to adhere to frequent oral feeds with a set volume goal (40–60 mL, every 2–3 h), leading to normoglycemia with the exception of one glucose to 44 mg/dL, which resolved with feeds. Lactic acid levels stabilized (3–6 mmol/L) but did not improve, and so he was placed on thiamine, riboflavin and biotin supplements (due to suspicion for mitochondrial condition). A post-prandial increase in glucose and lactate of >20% compared to pre-prandial levels was documented on two occasions with a lactate to pyruvate ratio of 23; however, this was not a consistent finding. Other notable laboratory findings included total bilirubin 4.8 mg/dL, direct bilirubin 1.63 mg/dL, INR of 1.3 with low fibrinogen and factor VII level, proteinuria, glycosuria and electrolyte wasting suggestive of renal tubular dysfunction. Transaminases, triglycerides, and uric acid levels were within normal limits. Radiologic evaluations were relevant for mild pelviectasis with echogenic pyramids bilaterally (abdominal ultrasound) and normal echocardiogram. Patient was placed on thiamine, riboflavin and biotin supplementation given persistent elevated lactate.

Rapid trio exome sequencing (ES) and mitochondrial genome sequencing was ordered through a commercial laboratory (GeneDx). Rapid ES revealed a *de novo* hemizygous pathogenic variant in *PHKA2* (c.3208_3210delGAG, p.R1072del), associated with the diagnosis of glycogen storage disorder (GSD) type IXα2 (Fig. 2). At one month of age, lactic acidosis appeared persistent and independent of prandial status

(6.3–11.9 mmol/L). Patient subsequently failed newborn hearing screen and was found to have bilateral high-frequency sensorineural hearing loss (SNHL). Mitochondrial genome sequencing was non-diagnostic but revealed a maternally inherited homoplasmic variant of uncertain significance (VUS) in *MT-CYB* (m.15534A>G, p.N263S); patient's mother was healthy with no hearing impairment. Given the findings of persistent lactic acidosis and SNHL, exome reanalysis was ordered and reported heterozygous VUSs in *CDH23*, *FASTKD2* and *SLC17A5* (Table 1). Follow-up deletion/duplication analysis for *CDH23*, *FASTKD2* and *SLC17A5*, as well as *PDH*, was negative.

Patient was discharged home with routine follow-up by multiple subspecialists. Repeat biochemical studies revealed decreased free carnitine (6.3 umol/L), elevated serum alanine (691 nmol/L), and elevations of lactic, malic, fumaric and pyroglutamic acid in urine. As an outpatient, he continued to demonstrate poor weight gain and failure to thrive, despite several different interventions to increase caloric intake (Fig. 3). At 3 months of age, patient required admission due to evidence of metabolic lactic acidosis with hypernatremia, hyperchloremia, wide anion gap, and transaminitis in the setting of febrile illness, diarrhea, and poor oral intake. Nephrology evaluation was suggestive of a proximal renal tubular acidosis (RTA) based on decreased tubular phosphate reabsorption (50%), generalized aminoaciduria, altered urine albumin/protein ratio (0.2), urine pH of 5 and low serum bicarbonate. Echocardiogram on admission showed dilated right ventricle with moderate-severely depressed systolic function and indirect findings suggestive of elevated right ventricle pressure; left ventricle compressed with hyperdynamic systolic function.

Infectious evaluation, including COVID-19 RT-PCR, returned non-diagnostic. Clinical and laboratory evidence suggested worsening liver function and coagulopathy with INR of 1.6 with low fibrinogen. Liver biopsy revealed macro- and micro-vesicular diffuse steatosis compatible with his diagnosis of GSD IXα2. Other findings included minimal lymphocytic portal inflammation with isolated hepatocellular necrosis and early pericellular fibrosis. Electron microscopy showed non-specific findings including moderate amount of glycogen and enlarged mitochondria. During this admission, patient developed worsening metabolic acidosis, respiratory failure and bradycardic arrest, ultimately resulting in his demise. Autopsy revealed biventricular hypertrophy, hepatomegaly (liver weight over two times expected weight for age) and interstitial pneumonitis with acute alveolar damage, consistent with acute viral pneumonia. Post-mortem mitochondrial genome reanalysis using a liver specimen was non-diagnostic (Baylor Genetics). The previously identified maternally inherited homoplasmic variant was reported as likely-benign and was found to be homoplasmic in the mother of the proband (Table 1). Both mother and sister underwent biochemical evaluation (ACP, PAA, UOA, serum carnitine, serum lactate) with

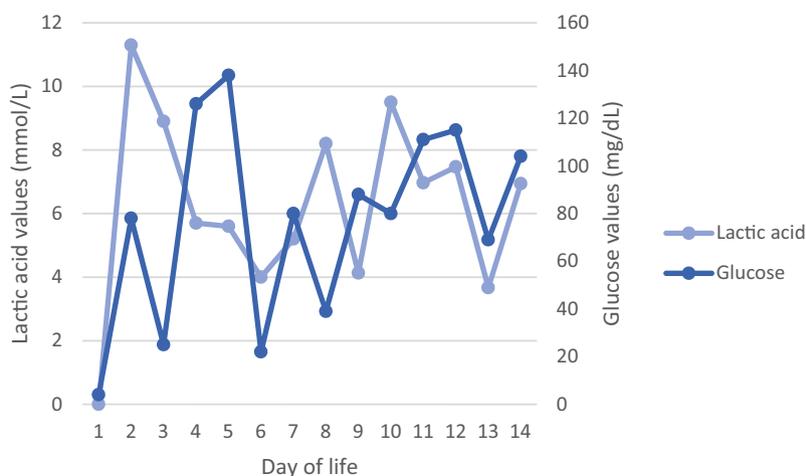


Fig. 1. Selected serum glucose and lactic acid levels during first 2 weeks of life.

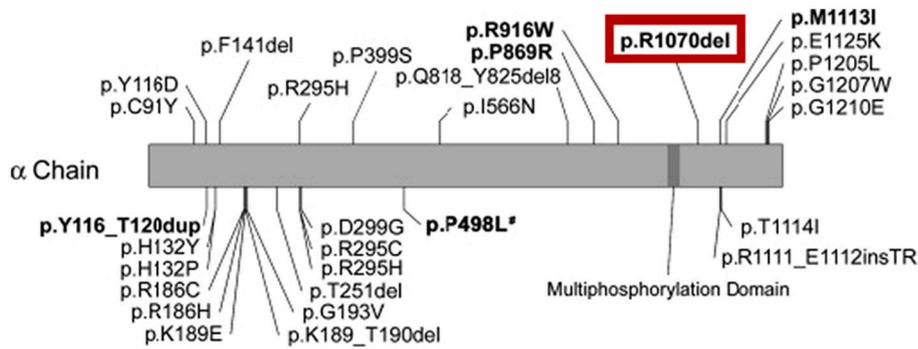


Fig. 2. Pathogenic variants in PHKA2, which encodes the α -subunit of the phosphorylase kinase (PhK) enzyme. Modified from Beauchamp et al. 2007. Red encasing denotes patient's variant.

Table 1
Nuclear and mitochondrial variants detected through whole exome sequencing.

Gene	Type	Inheritance	Variant	Zygosity or plasm	Classification
PHKA2	Nuclear	X-linked (<i>de novo</i>)	c.3210_3212delGAG (p.R1072del)	Hemizygous	Pathogenic
CDH23	Nuclear	AR (Paternal)	c.6654C>A (p.D2218E)	Heterozygous	Uncertain significance
FASTKD2	Nuclear	AR (Maternal)	c.527C>T (p.A176V)	Heterozygous	Uncertain significance
SLC17A5	Nuclear	AR (Maternal)	c.1341delG (p.T448PfsX54)	Heterozygous	Uncertain significance
MT-CYB	Mito.	Maternal	m.15534A>G (p.N263S)	Homoplasmic ^a	Uncertain significance, Likely-benign

^a Variant found to be homoplasmic in mother of proband. AR: autosomal recessive; Mito: mitochondrial.

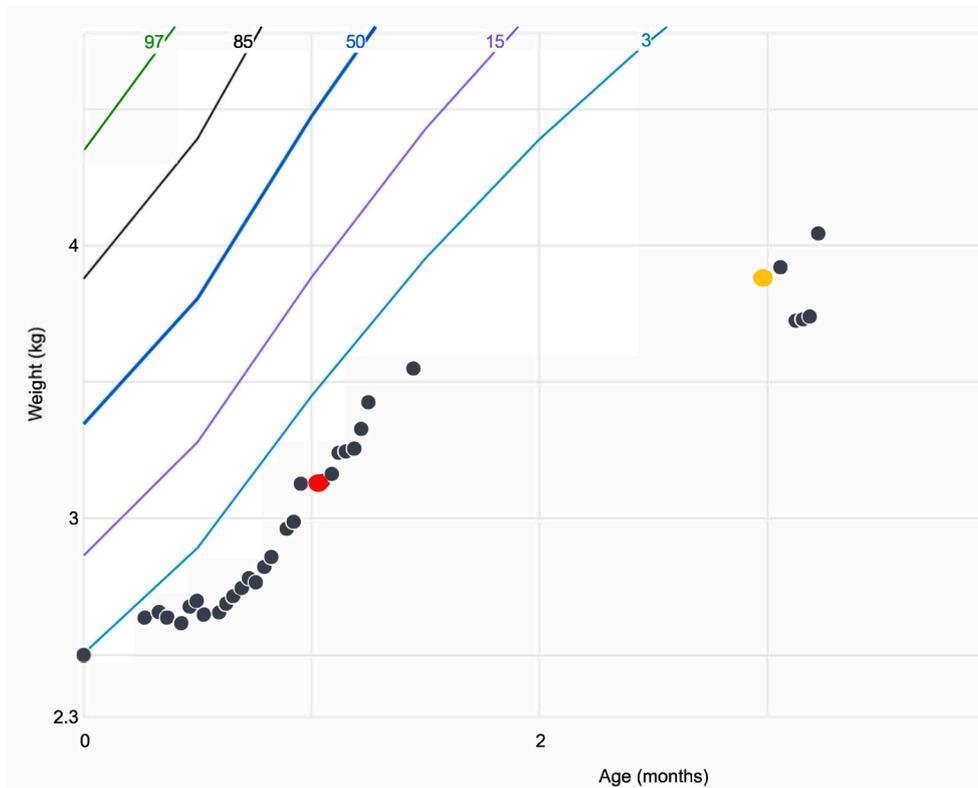


Fig. 3. Weight by age growth chart. Source: World Health Organization (WHO), 2006, Boys, birth to two years. Solid red circle: weight at time of discharge from birth admission. Solid yellow circle: weight at time of readmission.

normal results.

3. Discussion

Neonatal hypoglycemia is often a secondary finding in patients with

IUGR, SGA, or an underlying condition. Such conditions include hyperinsulinism, growth hormone deficiency, and inborn errors of metabolism (IEM) [11]. Specifically, hypoglycemia is a hallmark feature in GSDs, disorders of gluconeogenesis (GSD type 0), and fatty acid oxidation disorders (FAODs), primarily differentiated by the presence or

absence of ketones. Typically, GSDs present with ketotic hypoglycemia, while FAODs present with hypoketotic hypoglycemia.

This patient presented with mixed clinical and laboratory findings not diagnostic or consistent with any specific IEM. His ketotic hypoglycemia was suggestive of a GSD or disorder of gluconeogenesis; while the profound lactic acidosis favored PDH, mitochondrial disorder, FAOD, as well as some GSD subtypes due to severe hepatic dysfunction, as the liver is responsible for 70% of lactate clearance [12]. Initial improvement of the patient's hypoglycemia and lactic acidosis with IV dextrose and subsequent resolution of hypoglycemia after frequent oral feeding regimen, was suggestive of a GSD. More specifically, the post-prandial increase in glucose and lactate of >20% compared to pre-prandial levels was pointing to GSD 0 or PDH. In conjunction with an elevated lactate, a lactate to pyruvate ratio calculated as <25 (23 in our patient), indicates a defect in pyruvate metabolism, and is less suggestive of an oxidative phosphorylation or TCA cycle defect [13,14].

Ultimately, this patient's diagnosis was made through trio ES which revealed a *de novo* hemizygous pathogenic variant in *PHKA2* (c.3208_3210delGAG, p.R1072del), associated with GSD IX α 2 (Fig. 1). This variant has been reported in the literature in two other individuals: a 16 month-old boy from Finland with evidence of hepatomegaly, liver dysfunction and lactic aciduria, and a 4 year-old boy from South Korea presenting with hepatomegaly, short stature and lactic acidosis. Neither case had evidence of hypoglycemia [8,15]. These two cases, highlight the high variability seen, as while they share similar features to our patient (including lactic acidosis), their course of disease appeared to be milder than our case.

While post-prandial lactic acidosis has been reported in individuals with GSD IX α 2 [8,10], profound neonatal hypoglycemia with lactic acidosis has not been reported in patients with GSD IX α 2. In a cohort of 157 individual with GSD IX, only one individual presented with lactic acidosis (age and subtype not specified) [9]. A report of 26 individuals with molecularly-confirmed *PHKA2* pathogenic variants, the age of onset ranged from 3 to 36 months, 12-h fasting glucoses ranged from 43 to 84 mg/dL, and post-prandial lactatemia levels ranged from 2.6–8.1 mmol/L; lactic acidosis was not reported as a presenting feature [10]. Our patient's neonatal presentation is unusual for GSD IX α 2, as reported patients typically present after 3 months of age [9,10].

Hearing loss was not a reported feature in any of the aforementioned cohorts of patients with GSD IX [8–10]; however, bilateral postlingual SNHL has been reported in two brothers with GSD IX α 2 [16]. These brothers were diagnosed through ES and developed SNHL at the ages of 12 and 26 years, along with cognitive impairment and cerebellar involvement [16]. Hearing loss is a feature in other conditions with abnormal glycogen accumulation, such as Pompe disease, in which SNHL is more common among individuals with a severe infantile presentation compared to individuals with milder forms of the disease [17]. This has been evidenced by mouse models exhibiting abnormal glycogen storage in inner ear structures [18]. We question whether SNHL could be related to glycogen accumulation in a GSD IX α 2 context, *versus* a second unidentified etiology, as this case is the third individual with SNHL in this group of patients.

Proximal RTA is a feature associated with other GSDs, such as GSD I and GSD XI, but is not commonly described in GSD IX [1]. There are only a few published reports of individuals with GSD IX manifesting with renal tubulopathy. A 2.5 year-old boy diagnosed through low PhK enzyme activity, presented with doll-like face, growth delay, hepatomegaly, mild hypotonia and proximal RTA [19]. According to the authors, his growth and proximal RTA improved significantly, after administration of cornstarch therapy. He was later identified with two variants of interest in *PHKA2* (p.P399S and p.N953/L954I) [20]. It is unclear if these variants have been classified as pathogenic or benign, as we were not able to find them in public databases (ClinVar, gnomAD) [21]. The second case, consisted of a 12 year-old girl with GSD IXc due to a homozygous *PHKG2* pathogenic variant, who manifested renal tubular disease, considered secondary to rickets and inappropriate parathyroid

hormone response [8]. In our patient, no other etiology or comorbidity was identified. As renal tubular function is a process with great metabolic demand [22], GSD affected patients are at a potential disadvantage due to an abnormal glucose metabolism, perhaps leading to energy depletion in critical areas such as the kidney.

Lastly, our patient's severe presentation could be contradictory, as GSD IX α 2 is often considered more benign than other similar conditions like GSD IX γ 2 [9], but evidence of early onset liver pathology [9] suggests it could be a reality. The patient's persistent lactic acidosis, lack of correlation between lactate and glucose levels, age and severity of onset, hearing loss, and deteriorating clinical status were concerning for a mitochondrial disorder; however exome reanalysis and mitochondrial genome sequencing did not reveal another etiology for his condition beyond GSD IX α 2 at this time.

We hypothesize the cause of our patient's severe presentation, neonatal lactic acidosis and proximal RTA to be possibly associated to his GSD IX α 2 diagnosis, given his thorough genetic evaluation. Nevertheless, a second genetic etiology or an unidentified factor cannot be completely excluded, as current molecular testing (exome sequencing) yield is ~30% [23]. It is possible his atypical critical manifestation of GSD IX α 2 in the setting of significant illness and/or metabolic stress may have resulted in worsening liver and kidney injury. In conclusion, we present profound neonatal lactic acidosis, renal tubular acidosis and congenital SNHL in a patient with GSD IX α 2. As additional patients with GSD IX α 2 are identified, there is clinical and diagnostic value in the continued effort to further characterize the phenotypic variability of this condition in order to provide families and clinicians with improved insight regarding patient prognosis, surveillance, and medical management.

Credit author statement

J. Andres Morales & Christina G. Tise: conceptualization, methodology, writing of original draft, review & editing. Amrita Narang & Paul C. Grimm: review & editing. Gregory Enns & Chung Un Lee: supervision, review & editing.

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Declaration of Competing Interest

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