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# MUTATION IN THE GLYCOGEN PHOSPHORYLASE KINASE (PHKA2) GENE IN TWO PATIENTS FROM CARTAGENA DE INDIAS: REPORT OF THE FIRST CASES IN COLOMBIA

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## ABSTRACT

The glycogen storage disease (GSD) type IX is a rare disease of variable clinical severity that mainly affects the liver tissue. Individuals with hepatic phosphorylase b-kinase (PHK) deficiency due to mutation in the PHKA2 gene (GSD IXa) may present hepatomegaly with elevated serum transaminases, ketotic hypoglycemia, hyperlipidemia, and poor growth with considerable variation in clinical severity. To identify and describe the different clinical manifestations of two brothers with the same type of mutation in the PHKA2 gene (GSD IXa), which represent the first two cases described in Colombia. Two children were studied, from the city of Cartagena, who according to the symptoms, findings to the physical, clinical and biochemical examinations performed in the Biochemistry Laboratory of the University of Cartagena, had high suspicion that they had some glycogenosis In addition, genetic studies were carried out at the Center for the Diagnosis of Molecular Diseases (CEDEM) in Madrid-Spain, through massive sequencing analysis, bioinformatic analysis, bioinformatic analysis of mutations and confirmation by sequencing of Sanger. We present the cases of two siblings, who according to the symptomatology, clinical, biochemical and genetic tests carried out, get to diagnose and confirm that they suffer from GDS IXa with mutation c.919-2A> G in hemicigosis of the PHKA2 gene, the which represents a new variant of this gene. The development of the so-called next generation sequencing technologies (NGS), such as those used in this study, is currently the method of choice to confirm the diagnosis of GSD, avoiding the use of a test invasive as the liver biopsy.

**Keywords:** Type IX glycogen storage disease, Hepatomegaly, Hypoglycemia, PHKA2 gene mutation, Phosphorylase Kinase Deficiency

## I) INTRODUCTION

Glycogen storage diseases or "glycogenosis" (GSD) are a group of hereditary diseases caused by alterations in the enzymes involved in the metabolism of glycogen, producing its accumulation in various tissues (1). Hence, glycogenosis are classified according to the deficient enzyme and the affected tissue (2). The most common type of glycogen storage disease is GSD IX, with an incidence of 1:100,000 births (3). GSD type IX, due to glycogen-phosphorylase

kinase (PHK) deficiency, accounts for 25% of cases, the most frequent being the PHKA2 subtype (GSD IXa; MIM: 306000) with X-linked recessive inheritance (3, 4 , 5).

GSD-IX was first described in the medical literature in 1966 by Dr. Hug and colleagues, who reported a girl with liver PHK deficiency that was consistent with autosomal recessive inheritance. Later, similar individuals were described in the medical literature whose cases were more consistent with X-linked inheritance (6).

Phosphorylase Kinase (PHK) (EC 2.7.11.19), also called phosphorylase b kinase, is an enzyme that plays a key role in the regulation of glycogenolysis, since it is necessary for the activation of glycogen phosphorylase, the main regulatory enzyme in the glycogen breakdown. PHK is an enzyme composed of 16 subunits, with four each of alpha, beta, gamma, and delta types, and encoded by different genes. At least two different versions of PHK are formed from the subunits: one is more abundant in liver cells and the other in muscle cells (2). Mutations in the genes PHKA1 (phosphorylase regulatory subunit alpha 1) located on the long arm of the X chromosome (Xq12), PHKA2 (phosphorylase kinase regulatory subunit alpha 2) located on the short arm of the X chromosome (Xp22), PHKB (phosphorylase kinase regulatory subunit beta) located on the long arm of chromosome 16 (16q12) and PHKG2 (phosphorylase kinase catalytic subunit gamma 2) located on the short arm of chromosome 16 (16p11), are the most common subtypes and are classified as GDSIXd, GDSIXa, GDSIXb and GDSIXc, respectively. Mutations causing alterations in the Delta subunit (CALM1; 14q32) are linked to calcium regulation, but do not have clinical sequelae (4).

The protein encoded from the PHKA1 gene is a subunit of the muscle enzyme, while the protein encoded from the PHKA2 gene is part of the liver enzyme. Whether in the liver, or in the muscles, PHK plays an important role in providing energy for cells. The phenotype of these patients varies from mild forms with hepatomegaly and elevated liver enzymes, to more severe presentations with hypoglycemia, short stature, and mild gross motor retardation (7). Hypercholesterolemia, hypertriglyceridemia, and ketosis can also be observed after fasting (7,8,9). The symptoms generally improve with age, although in some cases liver fibrosis may appear and evolve into cirrhosis (4, 10).

## 2) MATERIALS AND METHODS

Two children were studied, from the Napoleón Franco Pareja Children's Hospital (Casa del Niño) in the city of Cartagena, in whom, according to the symptoms, findings of the physical and clinical examinations identified during the specialized consultation, it was suspected that they suffered from some glycogenosis. . After signing the informed consent, pathology studies, diagnostic images, biochemical analysis, and genetic studies were performed.

In relation to the genetic studies, massive sequencing analysis (Ilumina TruSight Panel and NextSeq 500), bioinformatic analysis (DNAnexus and VariantStudio), bioinformatic analysis of mutations (Alamut® Visual Interactive Biosoftware, Mutalyzer 2.0.14) and confirmation by Sanger sequencing (Bigdye v3.1 Applied Biosystems).

## 3) RESULTS AND DISCUSSION

We present the clinical cases of two brothers with clinical suspicion of glycogenosis, which, according to previous studies carried out in the Biochemistry Laboratory of the University of Cartagena, were mainly compatible with GSD types I, III, VI and IX.

The younger brother began at 11 months of age with abdominal distension, hepatomegaly of 5 cm below the right costal margin, nephromegaly,

short stature, cyanosis, and tonic-clonic seizures associated with vomiting. In addition, he presented with hypertransaminasemia, hypercholesterolemia, hypertriglyceridemia, and hyperuricemia along with episodes of hypoglycemia. A PAS-positive liver biopsy was performed, which was compatible with GDS, with glycogen of normal structure. A fractionated diet supplemented with cornstarch is implemented before going to sleep. At 5 years of age, he continues with persistent hematuria and asymptomatic intermittent hypoglycemia. At 9 years of age, he continued with hepatomegaly and nephromegaly, as well as a fracture of the left arm that did not heal well. At 11 years of age, the hepatomeglia had reduced to 2 cm, he presented short stature and body mass index in the lower normal limit, with a good cognitive level and academic performance according to his age. At 19 years of age, a genetic study was carried out confirming GDS IXa with mutation c.919-2A>G in hemizygosis of the PHKA2 gene.

The older brother is taken to the clinic at 18 months of age due to abdominal distension, hepatomegaly of 2 cm under the right costal margin, seizures, cyanosis and anasarca. The blood analysis showed cholesterol, triglycerides and uric acid in upper normal limits. He did not present hypoglycemia. At 5 years of age, a renal ultrasound was performed, which showed no alterations, normal neurological examination, normal physical examination, and with remission of symptoms. At 21 years of age, a genetic study was carried out that confirmed GDS IXa with the c.919-2A>G mutation in hemizygous PHKA2 gene, confirming that he presented the same mutation as his younger brother.

GSD IX is a significant subgroup of GSDs, however its biochemical and genetic diagnosis has been complicated by its spectrum of clinical symptoms, genetic heterogeneity, and phenotypic overlap with other GSDs (11). Thus, we see that there is a broad-spectrum phenotype associated with mutations in the PHKA2 gene responsible for GSD IXa, such as that observed in the patients in this study, where a mild presentation with hepatomegaly could be observed in the older brother, at a more severe presentation with hepatomegaly, nephromegaly, dyslipidemia, hypertransaminemia, hypoglycemia, and short stature in the younger sibling. The specific symptoms that develop and the overall severity of GSD-IX can vary greatly from individual to individual, even between individuals with the same subtype, as we have described in these two siblings.

The hypoglycemia associated with the disease is generally mild and does not always require treatment, except for the prevention of prolonged fasting periods, as well as the establishment of additional nocturnal feedings during infectious episodes (12).

This was observed in the younger brother who presented intermittent hypoglycemia, therefore, to maintain stable glucose levels, a divided diet rich in slow-absorbing carbohydrates was recommended and, as a complement, the administration of cornstarch prior to night rest. These recommendations were followed until they were approximately 7 years old. Apart from these measures, it is usually not necessary to impose additional restrictions on the patient's habits (12). As described in the literature, patients debut in early childhood, as in these two brothers, mainly with hepatomegaly, but also with delayed growth and mild delay in motor development. On occasions, cases of osteopenia with rounded cheeks were reported, so the risk of bone fracture may be higher (13), which could be observed in the younger brother who, at 9 years of age, presented a left arm fracture with poor consolidation.

In the form of the disease that affects the liver, as in these two cases, where the mutation is in the PHKA2 gene, the signs and symptoms generally improve with age. In general, these individuals reach their normal development and height when they are adults (14).

Therefore, GSD IXa is considered a benign condition, since patients may become asymptomatic as they grow older and treatment is often not necessary. However, recent studies have shown that untreated children may develop undesirable effects such as morning sickness, which could affect their school performance, and delayed growth, which can cause psychological distress (15).

In this study, we identified a new variant of the PHKA2 gene with mutation c.919-2A>G in hemizygosis in the two brothers, and the in silico analysis revealed the possible pathogenicity of this variant, although without in vitro functional analysis, it cannot be assured. that causes disease. The novel PHKA2 variant identified in this study confirms the diagnosis of GSD IXa, allowing differential diagnosis with respect to other GSD types (I, III, and VI) where the clinical manifestations overlap, except for the pattern of inheritance (16). As a result of this, genetic or molecular analysis is necessary to distinguish between these diseases, allowing for accurate diagnosis, where enzymology is not informative and identifies the pattern of inheritance, important for counseling and family studies (17, 18).

To date, the treatment of the disease refers to palliative therapies aimed at minimizing the incidence of symptoms, mainly based on appropriate nutritional guidelines (19). The use of these therapies varies significantly from one patient to another, and it is not uncommon to find children who evolve favorably spontaneously, without receiving any treatment, as happened with the older brother in this study.

Due to the relatively benign nature of this pathology, it is unlikely that therapies will emerge in the immediate future that allow for an effective cure of the disease, such as enzyme replacement therapy or gene therapies, since the high cost associated with the development and application of the in itself makes them economically unattractive for a rare disease such as GSD type IX (20). However, since this is a relatively common glycogenosis, we consider it essential to increase the degree of knowledge of it among the medical community in our country, in order to guarantee the generalization of a rapid and accurate diagnosis of the disease, in order to to put the available therapeutic guidelines into practice as soon as possible and prevent patients from suffering unnecessarily possible sequelae as a consequence of the lack of appropriate treatment for this pathology. Glycogenosis type IX should always be considered in the differential diagnosis in children with apparently asymptomatic chronic hepatomegaly.

## 4) CONCLUSION

The development of so-called next generation sequencing technologies (Next Generation Sequencing [NGS]), such as those used in this study, have made these the method of choice to confirm the diagnosis of GSD, replacing the quantification of glycogen content and to the determination of enzymatic activity, in this case of PHK, avoiding the use of an invasive test such as liver biopsy.

## 5) AUTHORSHIP CONTRIBUTIONS

All authors have jointly and equally contributed to the argumentation and writing of the manuscript.

## 6) FUNDING

None.

## 7) CONFLICT OF INTEREST

None.

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