



Short Communication

A new variant in *PHKA2* is associated with glycogen storage disease type IXa

Carmen Rodríguez-Jiménez^a, Fernando Santos-Simarro^{a,c}, Ángel Campos-Barros^{a,c}, Carmen Camarena^b, Dolores Lledín^b, Elena Vallespín^{a,c}, Ángela del Pozo^{a,c}, Rocío Mena^a, Pablo Lapunzina^{a,c}, Sonia Rodríguez-Nóvoa^{a,*}

^a Institute of Medical & Molecular Genetics (INGEMM), Hospital Universitario La Paz, Universidad Autónoma de Madrid, IdiPAZ, Madrid, Spain

^b Hepatology Unit, Hospital Universitario La Paz, Madrid, Spain

^c Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER, U753), Instituto Carlos III, Madrid, Spain

ARTICLE INFO

Article history:

Received 29 October 2016

Received in revised form 1 January 2017

Accepted 2 January 2017

Available online 12 January 2017

ABSTRACT

Glucogenosis type IX is caused by pathogenic variants of the *PHKA2* gene. Herein, we report a patient with clinical symptoms compatible with Glycogen Storage Disease type IXa. *PYGL*, *PHKA1*, *PHKA2*, *PHKB* and *PHKG2* genes were analyzed by Next Generation Sequencing (NGS). We identified the previously undescribed hemizygous missense variant NM_000292.2(*PHKA2*):c.1963G>A, p.(Glu655Lys) in *PHKA2* exon 18. *In silico* analyses showed two possible pathogenic consequences: it affects a highly conserved amino acid and disrupts the exon 18 canonical splice donor site. The variant was found as a “*de novo*” event.

© 2017 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Glycogen storage diseases (GSD) are a group of disorders caused by alteration of the enzymes involved in glycogen metabolism, producing an accumulation of glycogen in several tissues [1]. GSD are classified according to the enzyme deficiency and the affected tissue [2].

The most frequent type of glycogen storage disease is GSD IX, which incidence is 1:100,000 births and is responsible for 25% of all GSD cases [3]. GSD IX is caused by defects in the activity of the phosphorylase kinase complex (PHK, E.C. 2.7.1.38), which plays a critical role in regulating glucose release from glycogen, causing an excess of glycogen storage [4]. Patients with GSD IX generally present hepatomegaly, short stature, raised transaminases and triglycerides and sometimes ketotic hypoglycemia in early childhood. PHK is a decahexameric enzyme composed by four subunits: α , β , γ and δ . Subunits α and β have regulatory functions, the γ -subunit has catalytic function and δ -subunit is identical to Calmodulin. The phosphotransferase activity of PHK is Ca^{2+} dependent. Subunit α has two isoforms encoded by two different genes: *PHKA1* and *PHKA2*, which encode the muscular and liver isoforms, respectively. Subunits β , γ and δ are encoded by *PHKB*, *PHKG2* and *CALM1*, respectively. *PHKA1* and *PHKA2* deficiencies are X-linked, while *PHKB* and *PHKG2* deficiencies are inherited according to an autosomal recessive pattern.

The most common GSD IX subtype is the subtype IXa, caused by mutations in *PHKA2*, accounting for approximately 75% of cases [5]. GSD IXa has been split biochemically in two further types: IXa1 (GSD9A1) with loss of PHK enzyme activity in peripheral blood cells and liver tissue, and IXa2 (GSD9A2) with normal PHK activity in blood cells. The clinical presentation of both subtypes is similar.

During childhood, patients with GSD IXa, can develop growth retardation, hepatomegaly, and ketosis with or without hypoglycemia, increases in triglycerides, cholesterol and transaminases. The clinical manifestations of the disease ameliorate with age, being asymptomatic in adulthood [6]. Therefore, GSD IXa has been described as a benign form since often does not need treatment. However, genetic variants in *PHKA2* are associated with a broad phenotypic spectrum [7] and even cases of cirrhosis have been described. The first reported case was a white 18 months old boy who had micronodular liver cirrhosis and presented the mutation p.Arg298Pro [5]. Later reviews have described more patients with hepatic fibrosis at diagnosis who presented mutations in *PHKA2* [8]. Other uncommon phenotypes have been reported in the literature, such as kidney dysfunctions due to renal tubular acidosis and CNS affection with delayed cognitive and speech abilities as a result of *PHKA2* pathogenic variants [6].

To date The Human Gene Mutation Database (HGMD) has reported over 80 mutations in *PHKA2* associated with GSD IXa, including missense, splicing, small deletions, small insertions and gross deletions variants.

In this paper we describe a novel variant in *PHKA2* using Next Generation Sequencing (NGS).

* Corresponding author at: Genetics of Metabolic Diseases Laboratory-INGEMM, Hospital Universitario La Paz, Paseo de la Castellana 261, 28046 Madrid, Spain.
E-mail address: soniamaria.rodriguez@salud.madrid.org (S. Rodríguez-Nóvoa).

2. Case report

The patient of Spanish origin was born after 40 weeks and 5 days of gestation by normal vaginal delivery. He is the single child of healthy non-consanguineous parents. The paternal great-grandmother, two brothers of the great-grandmother and one sister of the grandmother had transaminase elevation. His birth weight was 3.450 kg and birth length 50 cm. From 8 months onwards failure to thrive was noted and at age one year a routine laboratory analysis revealed an increase in liver enzymes. Subsequent analysis showed: ALT 113 (10–49 U/L) and AST 134 (0–37 U/L). He showed hepatomegaly and, slight hyperechogenicity. A complete biochemical profile done at 2 years 5 months, revealed the following results: Glucose 52 mg/dL (60–100), total cholesterol (TC) 228 mg/dL (<200), HDL-c 26 mg/dL (>40), LDL-c 166 (<160), triglycerides (TG) 181 mg/dL (<150), lactate dehydrogenase 351 UI/L (110–295), AST 108 UI/L (15–60), ALT 77 UI/L (<45), gamma-GT 39 UI/L (3–22), creatine kinase (CK) 415 UI/L (<171), serum iron 73 µg/dL (100–250), VSG 40 mm/h (0–15), mean corpuscular volume 85,8 fl (70–85), mean corpuscular hemoglobin concentration 30,9 g/dL (31,2–36,0), high levels of the ketonic bodies: acetoacetate 1.46 mmol/L (0.00–0.10), beta-hydroxy-butyrate 2.92 mmol/L (0.00–0.20), and plasma lactate 1.92 mmol/L (0.50–1.80). The lysosomal enzymes activity in lymphocytes was within the normal range.

During follow-up the patient showed a normal appetite and diarrhea most weeks. He felt happy and was not moody. He got tired easily, was less active than other children and complained of muscle weakness. In view of the biochemical results and the lack of neurological or cardiological manifestations, GSD III was felt to be less likely, considering in the differential diagnosis a GSD VI or IX. Finally, and after informed consent from the family, genetic testing of the genes associated with GSD VI and GSD IX: *PYGL*, *PHKA1*, *PHKA2*, *PHKB* and *PHKG2* was arranged.

3. Methods

3.1. Genetic analysis by Next Generation Sequencing (NGS)

After DNA extraction the sample was analyzed by NGS using the Illumina TrueSight One (TSO) assay. Library preparation and exome enrichment steps were performed according to manufacturer's workflow and sequenced in a MiSeq system. The TSO assay covers 12 Mb of the human genome, containing 4813 clinically relevant genes. We chose a specific subset of genes for sequence analysis: *PYGL*, *PHKA1*, *PHKA2*, *PHKB* and *PHKG2*. Sanger sequencing was used to confirm the presence of the variants of interest.

3.2. Bioinformatic analysis

Bioinformatic analysis was performed using algorithms developed by our bioinformatic unit. Briefly, sequences were mapped to the *CRCh37/hg19* human reference sequence and data bases used for analysis were Human Gene Mutation Database (HGMD® Professional) (www.biobaseinternational.com/hgmd) from BIOBASE Corporation; the Human Gene Mutation Database, Online Mendelian Inheritance in Man (www.omim.org); Gene Tests (www.genetests.org). Variant annotation was carried out with Ensembl's variant Effect Predictor Tool and was based on the transcripts ENST00000216392 (*PYGL*), ENST00000339490 (*PHKA1*), ENST00000379942 (*PHKA2*), ENST00000323584 (*PHKB*), ENST00000563588 (*PHKG2*).

The *in silico* predictors of pathogenicity used were CADD (Combined Annotation Dependent Depletion), Polyphen (Polymorphism Phenotyping), MutAssesor, Fasthmm and Vest. Scores of conservation used: Gerp2, PhasCons, Phylop, MaxEntScan, NNSplice, GeneSplicer and Human Splicing Finder were used as splicing predictors. Variants with minor allele frequency > 1% were excluded from further analysis. The files were uploaded in BAM format for analysis using Alamut Visual V.2.8.0 (Interactive Biosoftware; France).

4. Results

We identified the previously undescribed hemizygous missense variant NM_000292.2(*PHKA2*):c.1963G>A, p.(Glu655Lys) in *PHKA2* exon 18. The presence of the variant was confirmed by Sanger sequencing (Fig. 1). *In silico* analysis showed two possible consequences of this change:

First, a missense change in the amino acid Glutamate to Lysine in position 655 of the protein p.(Glu655Lys). Two out of five predictors of pathogenicity classified this variant as damaging or possible damaging (Fasthmm – 2.68 ($s < -1$ damaging) and VEST 0.597) and three further predictors classified it as benign (CADD 6.9 ($s < 11$ benign), Polyphen 0.01 ($s < 0.03$ benign), MutAssesor 0.415 ($s < 1.12$ benign). All proved conservation: Gerp2 5.78 (> 2.45 conserved), PhastCons (placenta) 0.96 (range 0–1), PhastCons (vertebrate) 1 (range 0–1), PhyloP (placenta) 2.5620 (score ≤ 2) and PhyloP (vertebrate) 3.3290 (score ≤ 2).

Second, since the mutation affects the last coding nucleotide of *PHKA2* exon 18; the splicing predictors MaxEntScan, NNSPLICE, GeneSplicer, Human Splicing Finder-like revealed that this variant may disrupt canonical splice donor site of exon 18, probably resulting in an aberrant protein. Splicing predictions at nearest natural junction predicted a decreased splicing efficiency at the canonical donor site by –68,9%. The variant was absent in the mother, thus, it is likely to have arisen as “*de novo*” event.

5. Discussion

GSD IX is considered as a benign condition, due to the fact that the patients may become asymptomatic when they grow up and frequently the treatment is not necessary [9]. However, recent studies have shown that untreated children may develop undesirable effects such as morning sickness, which could affect their school performance, and growth delay, which may result in psychological distress [10]. In adult patients the risk of bone fracture may be higher due to the reduced bone density secondary to delayed puberty [11]. In this study we identified a novel *PHKA2* variant in a patient with clinical suspicious of GSD. The patient showed a wide range of associated clinical features: hepatomegaly, growth delay, muscle weakness, fasting ketosis and high levels of liver enzymes and total cholesterol. These findings are consistent with the diagnosis of GSD IX. Genetic analysis of *PHKA2* identified a hemizygous missense variant NM_000292.2(*PHKA2*):c.1963G>A, p.(Glu655Lys) in exon 18, which could explain this phenotype. *In silico* analysis revealed the possible pathogenicity of this variant. Bioinformatics tools for predicting splicing effects showed that this variant disrupts exon 18 canonical splice donor site, probably resulting in an aberrant protein. According to the ACMG criteria for variant classification we classify this variant as pathogenic.

GSD IX is a significant subgroup of GSDs, however its biochemical and genetic diagnosis has been complicated due to its genetic heterogeneity and phenotypic overlapping with others GSDs [12]. Normoglycemic ketonemia is a usual biochemical feature in patients with GSD types VI and IX, and ketonemia can occur with hypoglycemia in all GSD types. Therefore, GSD VI and GSD IX must be incorporated into the differential diagnosis of ketotic normoglycemia, and KB (KB is the sum of blood acetoacetate and β -hydroxybutyrate concentrations) must be regularly measured in ketotic GSD patients. Also the clinical manifestations of GSD IX practically overlap with those of GSD VI except for the inheritance pattern. As a result of this, the genetic analysis is necessary in order to distinguish between these diseases [9]. The novel *PHKA2* variant identified in this study confirms the diagnosis of GSD IX allowing the differential diagnosis with regard to other types of GSD.

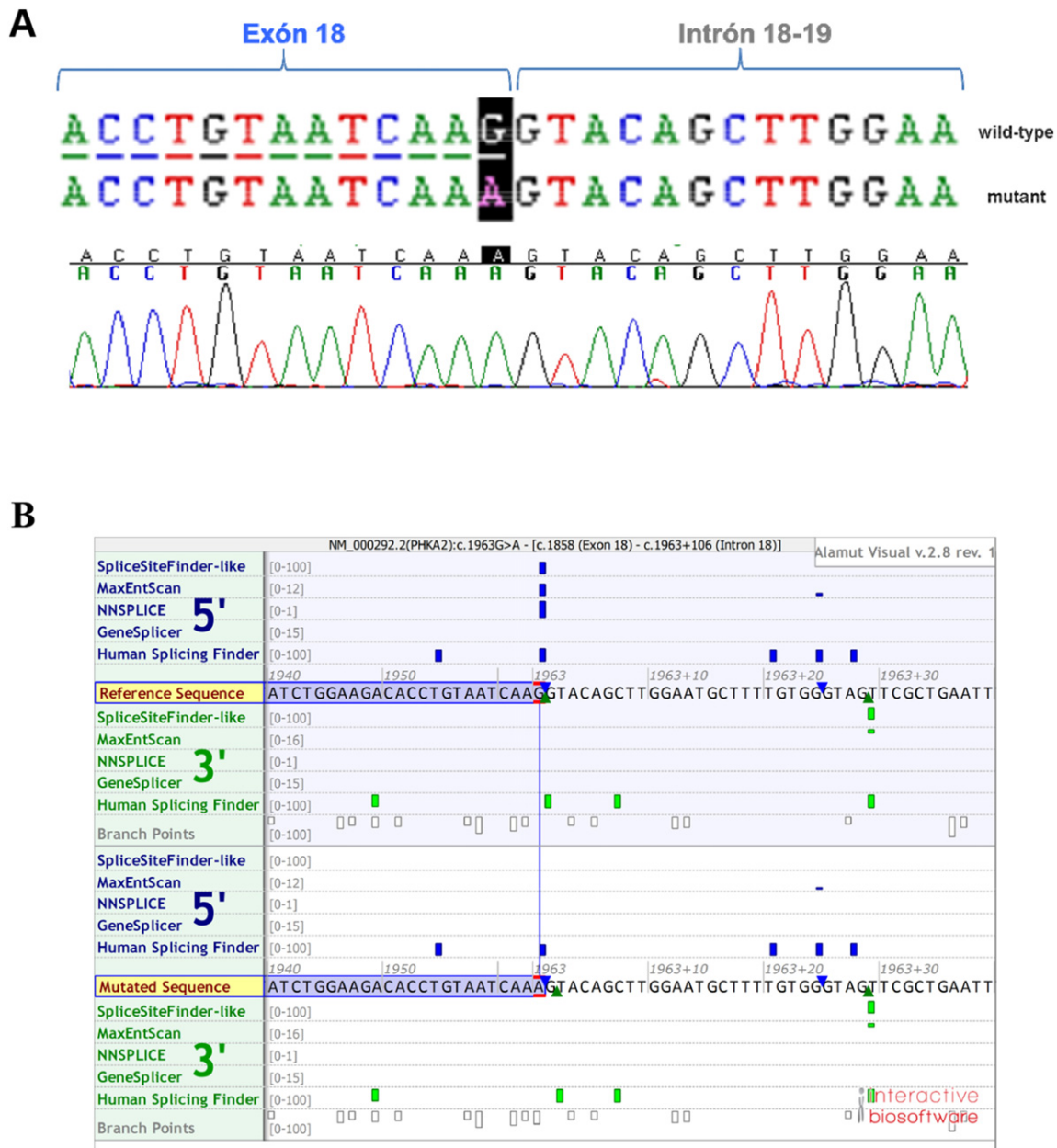


Fig. 1. Novel mutation in *PHKA2* (NM_000292.2(PHKA2):c.1963G>A, p.(Glu655Lys) in exon 18): (A) nucleotide sequence confirmation of the genetic variant, the transition from G to A at nucleotide position 1963 causes the change of Glutamic to Lysine at amino acid position 655; (B) AlamutVisual Splicing predictors (SpliceSiteFinder-like, MaxEntScan, NNSPLICE and GeneSplicer) indicate that the mutation may cause the loss of exon 18 splicing donor.

PHKA2 missense mutations can affect the N-terminal glucoamylase domain or the C-terminal calcineurin B-like domain. Variants associated with N-terminal domain principally lead to GSDIXa2 and are clustered into the predicted active site of the PHK α . They might have a direct impact on the predicted hydrolytic activity of the α subunit. The variants at the C-terminal domain lead to GSDIXa1 and are clustered in the region predicted to interact with the regulatory domain of the catalytic subunit in a region covering this interaction site leading to unstable PHK complex or less regulated holoenzyme [13]. In our patient, it was not possible to distinguish between GSDIXa1 and GSDIXa2 since the enzymatic assays was not available. Moreover, we cannot predict whether the variant c.1963G>A, p.Glu655Lys is affecting N-terminus or C-terminus domain. We can conclude that all *in silico* tools predict a deleterious impact on protein function and genetic test helps avoiding the use of much more invasive techniques such as quantification of glycogen content and PKH activity in liver biopsy samples.

References

- [1] C.K. Lau, J. Hui, F.N. Fong, K.F. To, T.F. Fok, N.L. Tang, et al., Novel mutations in *PHKA2* gene in glycogen storage disease type IX patients from Hong Kong, China, *Mol. Genet. Metab.* 102 (2) (2011) 222–225 (Feb).
- [2] K.P. Chen Y-T, D. Koeberl, Glycogen storage diseases, in: A.L.V.B. Beaudet, K.W. Kinzler (Eds.), *The Online Metabolic and Molecular Bases of Inherited Disease*, The McGraw-Hill Companies, Inc., New York, NY, 2013 (2013).
- [3] A.J. Maichele, B. Burwinkel, I. Maire, O. Sovik, M.W. Kilimann, Mutations in the testis/liver isoform of the phosphorylase kinase gamma subunit (*PHKG2*) cause autosomal liver glycogenosis in the *gsd* rat and in humans, *Nat. Genet.* 14 (3) (1996) 337–340 (Nov).
- [4] E. Mayatepek, B. Hoffmann, T. Meissner, Inborn errors of carbohydrate metabolism, *Best Pract. Res. Clin. Gastroenterol.* 24 (5) (2010) 607–618 (Oct).
- [5] A.O. Johnson, J.L. Goldstein, D. Bali, Glycogen storage disease type IX: novel *PHKA2* missense mutation and cirrhosis, *J. Pediatr. Gastroenterol. Nutr.* 55 (1) (2012) 90–92 (Jul).
- [6] B. Burwinkel, L. Amat, R.G. Gray, N. Matsuo, K. Muroya, K. Narisawa, et al., Variability of biochemical and clinical phenotype in X-linked liver glycogenosis with mutations in the phosphorylase kinase *PHKA2* gene, *Hum. Genet.* 102 (4) (1998) 423–429 (Apr).

- [7] N.J. Beauchamp, A. Dalton, U. Ramaswami, H. Niinikoski, K. Mention, P. Kenny, et al., Glycogen storage disease type IX: high variability in clinical phenotype, *Mol. Genet. Metab.* 92 (1–2) (2007) 88–99 (Sep–Oct).
- [8] L.A. Tsilianidis, L.M. Fiske, S. Siegel, C. Lumpkin, K. Hoyt, M. Wasserstein, et al., Aggressive therapy improves cirrhosis in glycogen storage disease type IX, *Mol. Genet. Metab.* 109 (2) (2013) 179–182 (Jun).
- [9] P. Burda, M. Hochuli, Hepatic glycogen storage disorders: what have we learned in recent years? *Curr. Opin. Clin. Nutr. Metab. Care* 18 (4) (2015) 415–421 (Jul).
- [10] H.M. Schippers, G.P. Smit, J.P. Rake, G. Visser, Characteristic growth pattern in male X-linked phosphorylase-b kinase deficiency (GSD IX), *J. Inherit. Metab. Dis.* 26 (1) (2003) 43–47.
- [11] J.S. Finkelstein, A. Klibanski, R.M. Neer, A longitudinal evaluation of bone mineral density in adult men with histories of delayed puberty, *J. Clin. Endocrinol. Metab.* 81 (3) (1996) 1152–1155 (Mar).
- [12] J.A. Kim, J.H. Kim, B.H. Lee, G.H. Kim, Y.S. Shin, H.W. Yoo, et al., Clinical, biochemical, and genetic characterization of glycogen storage type IX in a child with asymptomatic hepatomegaly, *Pediatr. Gastroenterol. Hepatol. Nutr.* 18 (2) (2015) 138–143 (Jun).
- [13] C. Carrière, S. Jonic, J.P. Mornon, I. Callebaut, 3D mapping of glycogenosis-causing mutations in the large regulatory alpha subunit of phosphorylase kinase, *Biochim. Biophys. Acta* 2008 (1782) 664–670.