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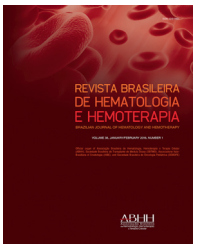

Original article

Novel mutations associated with pyruvate kinase deficiency in Brazil

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ABSTRACT

Background: Pyruvate kinase deficiency is a hereditary disease that affects the glycolytic pathway of the red blood cell, causing nonspherocytic hemolytic anemia. The disease is transmitted as an autosomal recessive trait and shows a marked variability in clinical expression. This study reports on the molecular characterization of ten Brazilian pyruvate kinase-deficient patients and the genotype–phenotype correlations.

Method: Sanger sequencing and *in silico* analysis were carried out to identify and characterize the genetic mutations. A non-affected group of Brazilian individuals were also screened for the most commonly reported variants (c.1456C>T and c.1529G>A).

Results: Ten different variants were identified in the *PKLR* gene, of which three are reported here for the first time: p.Leu61Gln, p.Ala137Val and p.Ala428Thr. All the three missense variants involve conserved amino acids, providing a rationale for the observed enzyme deficiency. The allelic frequency of c.1456C>T was 0.1% and the 1529G>A variant was not found.

Conclusion: This is the first comprehensive report on molecular characterization of pyruvate kinase deficiency from South America. The results allowed us to correlate the severity of the clinical phenotype with the identified variants.

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Introduction

Pyruvate kinase (PK) deficiency is the most common enzymatic defect of the glycolytic pathway, causing hereditary nonspherocytic hemolytic anemia. The prevalence of PK deficiency has been estimated to be 1:20 000 in the general white population.[1] The disease is caused by mutations in the *PKLR* gene, which are transmitted as an autosomal recessive trait with affected individuals being either homozygotes or compound heterozygotes. The most commonly reported mutations are missense variants, including c.1529G>A in the United States and Northern/Central Europe, c.1456C>T in Southern Europe, and c.1468C>T in Asia.[2–7]

The severity of anemia is quite variable, ranging from mild or fully compensated forms to life-threatening neonatal anemia that requires continuous transfusions. Other clinical features include jaundice, splenomegaly and gallstones in some patients.[8]

This study reports on the molecular analysis and the clinical description of PK deficient patients of Brazilian origin.

Methods

Patients

This study involved ten unrelated patients with PK deficiency originating from Southern Brazil. Patients were either diagnosed at the study center or diagnosed elsewhere and referred to this center for confirmation of the diagnosis and/or to establish the molecular basis of their PK deficiency. Other causes of hemolytic anemia were ruled out in all patients. Appropriate informed consent was obtained either directly, if the patients were over 12 years of age, or from their parents or guardians. The diagnosis was based on clinical history, hematological data, and demonstration of reduced PK activity in red blood cells.

Eight patients (A–H) displayed a severe phenotype (hemoglobin levels lower than 8 g/dL and/or transfusion dependence and/or splenectomy. Two patients (I and J) displayed a moderate-to-mild phenotype defined by hemoglobin greater than 8 g/dL, fewer than five transfusions and no splenectomy (Table 1).

Five hundred healthy blood donors from the Blood Center of the Universidade Estadual de Campinas were also studied to analyze the frequency of the c.1456C>T and c.1529G>A variants in a sample Brazilian population.

DNA and structural analysis

Venous blood was used for hematological and DNA analysis. Hematological parameters including red blood cell indices were investigated and a reticulocyte count was performed. PK enzyme activity and other biochemical determinations were carried out according to standard methods.[9]

Total genomic DNA was isolated from peripheral blood leukocytes by the standard salting-out method. Individual exons of the *PKLR* gene were amplified by polymerase chain reaction (PCR) and DNA sequence analysis was performed using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an ABI 3500xL Genetic Analyzer (Applied Biosystems) with the same primers used in the PCR reactions. The Chromas Lite 2.0 (Technelysium Pty Ltd) and CLC Sequence Viewer v.6.8.1 free software (CLC bio) were used to analyze and compare sequences with the reference *PKLR* sequence. Structural analyses were performed using the PDB ID: 2VGB – chain A as a template.[10] The native and mutant models were constructed by the SWISS MODEL web-served program.[11] Internal contacts were evaluated by STING Millennium[12] and 3D protein structures were generated using PyMOL (the PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC).

To study the frequency of the c.1529G>A and c.1456C>T variants, exon 11 of the *PKLR* gene was amplified by PCR. The c.1529G>A variant was screened using the StyI enzyme, which only digests mutated fragments, producing fragments of 144 and 112 base pairs (bp). The c.1456C>T variant was screened using the BsmAI enzyme that only cleaves the DNA of patients without these mutations resulting in fragments of 220 and 36 bp. The reactions were incubated in 37 °C for 16 h according to the manufacturer's recommendations and the products were subjected to agarose gel electrophoresis.

Table 1 – Hematological parameters of Brazilian patients with hemolytic anemia due to PK deficiency.

Patient	Sex	Age at presentation	Hb (g/dL)	Reticulocytes (% of RBC)	PK activity	Transfusions	Splenectomy
A	M	–	12.0	6.4	20′[a]	No	No
B	M	Newborn	7.0	–	6.5	Frequent	Yes
C	F	Newborn	8.0	4	9.7	Frequent	Yes
D	F	Newborn	7.3	6.21	25′[a]	Frequent	–
E	M	Newborn	10.5	2.5	3.8	Yes	No
F	F	Newborn	7.7	50	50′[a]	Yes	Yes
G	F	Newborn	4.8	25.4	11.7	Frequent	Yes
H	M	Newborn	5.9	1.2	1.65	Frequent	Yes
I	F	–	5.6	–	25′[a]	Frequent	Yes
J	M	7 yr.	8.5	12	45′[a]	Yes	Yes
Ref. values			12.2–16.7	0.5–2.0%	11.1–15.5 UI/gHb/min		

[a] Fluorescence of NADPH (Ref. value ≤15′).
–: no information.

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