



## Scientific Comment

# Pyruvate kinase deficiency: novel mutations and a better understanding of the genotype-to-phenotype correlation in Brazilian patients<sup>☆</sup>



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Over the past few years, the inherited disorders of erythrocyte metabolism have been the object of intensive research resulting in a better understanding of their molecular basis.<sup>1</sup>

Pyruvate kinase (PK) deficiency is the most common cause of congenital non-spherocytic chronic hemolytic anemia and is the result of an erythrocyte enzyme defect. It is an autosomal recessive condition caused by a deficiency of erythrocytic PK. Although the gene frequency for PK deficiency is far lower than that for glucose-6-phosphate dehydrogenase (G6PD) deficiency, the vast majority of patients inheriting G6PD deficiency never suffer acute or chronic hemolysis, whereas chronic hemolysis of variable severity is common in those with PK deficiency.<sup>1,2</sup>

PK deficiency is an extremely rare disorder; the prevalence of this deficiency is unknown. The PK-catalyzed reaction is the second ATP-generating step of the glycolytic pathway and is of particular importance in energy production, yielding nearly 50% of the total ATP. PK enzymes consist of several isoforms. They are products of two distinct genes: the M (muscle) gene is expressed in muscles, the brain, white blood cells, and platelets; it is located on chromosome 15q22 and the M1 and M2 isoforms are the result of a differential processing of this single gene transcript; and the LR gene on chromosome

1q21 with the isoforms L (liver) and R (red cell). Both genes encode an enzyme that catalyzes the transphosphorylation of phosphoenolpyruvate into pyruvate and ATP.<sup>1-3</sup> Clinical PK deficiency is limited to mutations of the LR gene observed in homozygous and compound heterozygous patients. Although abnormalities in the LR gene may result in alterations of both erythrocyte and liver enzymes, clinical symptoms are confined to red blood cells with hemolytic anemia; the hepatic deficiency is usually compensated by persistent enzyme synthesis in hepatocytes.<sup>3,4</sup>

The severity of hemolysis in PK-deficient patients is highly variable. It may be the cause of in-utero death from non-immune hydrops fetalis or death shortly after birth. However, more commonly, the disorder ranges from a life-threatening transfusion-dependent hemolytic anemia since birth to a mild, fully compensated hemolytic process.<sup>1,3,5</sup>

Patients with PK deficiency and severe hemolysis usually present with pallor, icterus, and splenomegaly, and develop the clinical complications typical of chronic hemolytic states. These complications include varying degrees of hepatosplenomegaly, pigmented (bilirubin) gallstones, iron overload (can be severe even in non-transfused patients and clinically significant, especially in those requiring

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multiple transfusions), worsening anemia secondary to transient aplastic anemia caused by infection (e.g., parvovirus), folic acid deficiency due to increased requirements, skin ulcers, and worsening hemolysis during pregnancy and after the use of oral contraceptives.<sup>1,3,5</sup>

Individuals with PK deficiency uniformly have evidence for increased red blood cell destruction, including elevated levels of indirect bilirubin. Hemolysis is mainly extravascular; however, elevations of serum lactate dehydrogenase and reduced levels of haptoglobin may be variably seen.<sup>1,3,5</sup>

Examination of the peripheral smear most often demonstrates morphologic abnormalities (increased polychromasia, reticulocytosis and contracted echinocytes in some patients); however, these changes are nonspecific and not consistently observed in PK-deficient patients. Unlike congenital spherocytic hemolytic anemias, osmotic fragility of the patient's red blood cells is normal.<sup>1,3,5</sup>

No laboratory measurement other than an assay of erythrocytic PK enzymatic activity is helpful in arriving at an appropriate diagnosis. High levels of the glycolytic intermediate 2,3-BPG are common in PK deficiency but it is non-specific.

Testing for PK deficiency is appropriate in any sibling of a patient with PK deficiency who has unexplained anemia, and in any individual with suspected congenital hemolytic anemia with a negative direct antiglobulin test and absence of morphologic abnormalities suggestive of other conditions (e.g., absence of spherocytes, basophilic stippling and Heinz bodies).<sup>1,3,5</sup>

In addition to the PK activity test, the mutant PK enzyme may also be analyzed by the detection of PK LR gene mutations at the cDNA or genomic level.<sup>6</sup> These latter tests also allow for prenatal testing. The determination of the causative mutation(s) and their presence in parents is essential for cases in which prenatal diagnosis is used.

The diagnosis of PK deficiency is made when there is clinical or laboratory evidence for hemolytic anemia and one or both of the following: low levels of erythrocytic PK enzymatic activity and presence of the PK LR gene mutation that would be expected to impair enzyme activity.<sup>1,2,6</sup>

So far, no specific therapy for PK deficiency is available, and the treatment of this disease is, therefore, based on supportive measures such as: frequent blood transfusions and splenectomy that usually results in an increase of 1–3 g/dL in hemoglobin thereby reducing or even eliminating transfusion requirements in most transfusion-dependent cases. Hyperbilirubinemia during the neonatal period may necessitate phototherapy or exchange transfusion, and iron chelation may be required since iron overload is rather common in PK deficiency, even in non-transfused patients.<sup>1-3,7</sup>

In patients with extremely severe disease who continue to be transfusion-dependent after splenectomy,

allogeneic hematopoietic stem cell transplantation may be considered.<sup>1</sup>

In this issue of the Brazilian Journal of Hematology and Hemotherapy, Svidnicki et al. report on the molecular characterization of ten Brazilian PK-deficient patients and its correlation with the clinical phenotype severity with the identified variants.<sup>8</sup> They also studied a non-affected group of Brazilian individuals screened for the most commonly reported variants. Ten different variants of the PK LR gene were identified, 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 us with a rationale for the observed enzyme deficiency.

At present, although the number of cases in which the mutations are known has expanded rapidly, the number of cases in which clinical manifestations are also described is still quite limited. This is the first comprehensive report of the molecular characterization of PK deficiency from South America and its data contribute to a better understanding of the genotype-to-phenotype correlation in PK deficiency.

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## Conflicts of interest

The author declares no conflicts of interest.

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