Case Report

Pyruvate Kinase Deficiency presented with Severe Anemia and Jaundice

Arunothai Rakmanotham, Darintr Sosothikul, Piti Techavichit, Supanun Lauhasurayotin, Kanhatai Chiengthong and Hansamon Poparn

Clinical Research for Holistic Management in Pediatric Hematology and Oncology, Department of Pediatrics, Faculty of Medicine, King Chulalongkorn Memorial Hospital, Chulalongkorn University

Abstract:

Pyruvate kinase, an enzyme in the glycolytic pathway of red blood cells, plays an important role in producing energy or ATP for red blood cells. Pyruvate kinase deficiency is a rare hereditary red cell disorder caused by mutation in the Pyruvate Kinase L/R (PKLR) gene on chromosome 1q12. Homozygous or compound heterozygous mutation in the PKLR gene can cause nonspherocytic hemolytic anemia due to lack of red cell ATP, leading to inability to maintain red cell membrane integrity and electrochemical gradients. We report clinical presentations, laboratory investigations and genetic testing for diagnosis and management of a 1-year-old Thai girl with a history of severe nonspherocytic hemolytic anemia and neonatal hyperbilirubinemia, requiring an exchange transfusion, at 24 hours of life. She received a regular red cell transfusion since day-of-life 2 and was subsequently diagnosed with pyruvate kinase deficiency.

Keywords : ● *Pyruvate kinase deficiency* ● *Nonspherocytic hemolytic anemia* ● *PKLR mutation*

J Hematol Transfus Med. 2021;31:369-76.

Received 1 November 2021 Corrected 9 November 2021 Accepted 1 December 2021

Correspondence should be addressed to Hansamon Poparn, MD., Clinical Research for Holistic Management in Pediatric Hematology and Oncology, Department of Pediatrics, Faculty of Medicine, King Chulalongkorn Memorial Hospital, Chulalongkorn University, Bangkok 10330
E-mail: hansamonp@gmail.com

รายงานผู้ป่วย

ภาวะพร่องเอนไซม์ไพรูเวทไคเนสที่มาด้วยอาการซีดและตัวเหลืองอย่างรุนแรง

อรุโณทัย รักมโนธรรม ดารินทร์ ซอโสตถิกุล ปิติ เตชะวิจิตร สุภานัน เลาหสุรโยธิน กาญจน์หทัย เชียงทอง และ หรรษมน โพธิ์ผ่าน

หน่วยโรคเลือดและมะเร็งในเด็ก ภาควิชากุมารเวชศาสตร์ คณะแพทยศาสตร์ โรงพยาบาลจุฬาลงกรณ์

บทคัดย่อ

เอนไซม์ pyruvate kinase เป็นเอนไซม์ที่สำคัญของเม็ดเลือดแดงที่ใช้ใน glycolytic pathway ซึ่งเป็นกระบวนการสร้างพลังงานหรือการสร้าง ATP ของเม็ดเลือดแดง โรคภาวะพร่องเอนไซม์ pyruvate kinase (pyruvate kinase deficiency) เป็นโรคความผิดปกติแต่กำเนิดของเม็ดเลือดแดงที่พบได้น้อย เป็นโรคที่ทำให้เกิดภาวะซีดจากการที่เม็ดเลือดแดงแตกง่าย สาเหตุเกิดจากความผิดปกติทางพันธุกรรมที่มีการกลายพันธุ์บริเวณยีน PKLR บนโครโมโซมคู่ที่ 1 โดยมีการถ่ายทอดทางพันธุกรรมในลักษณะยีนด้อย ผู้ป่วยที่มีความผิดปกตินี้มีได้ตั้งแต่ไม่มีอาการ ไปจนถึงมีอาการรุนแรงจนมีอาการซีดจากเม็ดเลือดแดงแตก ตัวเหลืองตั้งแต่แรกเกิดจนอาจต้องรับการเปลี่ยนถ่ายเลือด และต้องได้รับเลือดอย่างสม่ำเสมอ ในรายงานผู้ป่วยฉบับนี้จะกล่าวถึงอาการ การวินิจฉัย การตรวจทางห้องปฏิบัติการ การตรวจทางพันธุศาสตร์ และการรักษา ในผู้ป่วยเด็กหญิงอายุ 1 ปี ที่มีประวัติซีดร่วมกับตัวเหลืองจนต้องเปลี่ยนถ่ายเลือดตอนอายุ 24 ชั่วโมงแรก ซึ่งภายหลังพบการกลายพันธุ์ของยีนPKLR และได้รับการวินิจฉัยเป็น โรคภาวะพร่องเอนไซม์ pyruvate kinase (pyruvate kinase deficiency)

คำสำคัญ : ● *ภาวะพร่องเอนไซม์ไพรูเวทไคเนส* ● *ภาวะซีดจากเม็ดเลือดแดงแตก* ● *การกลายพันธุ์ของยีน PKLR*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2564;31:369-76.

Introduction

Pyruvate kinase deficiency (PKD) is a rare red cell disorder with the estimated prevalence of the disease among Caucasians around 1 in 20,000 population[1]. PKD is a hereditary disease involving defects in the pyruvate kinase enzyme in the glycolytic pathway of red blood cells and causes chronic nonspherocytic hemolytic anemia[1,2]. Mature red blood cells (RBCs), lacking of a nucleus and organelles like ribosomes or mitochondria, have two major pathways: the glycolytic or "energy-producing" pathway and the hexose monophosphate (HMP) shunt or "protective" pathway[1]. Pyruvate kinase (PK) catalyzes phosphoenolpyruvate to pyruvate, the final steps of the glycolytic pathway, to create 50% of total RBC adenosine triphosphate (ATP)[2,3]. Due to the lack of RBC ATP, red blood cells lose their ability to maintain membrane integrity and electrochemical gradients leading to extravascular hemolysis from clearance of damaged RBCs[3-5].

This defect is inherited by the autosomal recessive pattern involving homozygotes or compound heterozygotes in the PKLR gene located on chromosome 1q21[2,4]. PKD is a lifelong chronic hemolytic anemia with a wide spectrum of symptoms, manifestations, and complications. In most cases, the hemolytic process is recognized and diagnosed in childhood with history of neonatal jaundice requiring phototherapy or an exchange transfusion.

Enzymopathies, such as pyruvate kinase deficiency, should be suspected among patients of all ages with chronic hemolytic anemia in the absence of immune-mediated hemolysis, hemoglobinopathy, or evidence of a red cell membrane disorder. Among many patients, direct enzyme analysis is adequate for initial diagnosis, with molecular testing serving as a confirmatory test[1].

Case presentation

A 1-year-old Thai girl, the third child of nonconsanguineous parents, was delivered by normal delivery at 37 weeks of gestation in a provincial hospital with Apgar scores 7, 7 and 9 at 1, 5 and 10 minutes after birth respectively. Her prenatal history was uneventful with a birth weight of 2.28 kg. After birth, she was resuscitated and intubated for seven days due to acute respiratory failure and meconium aspiration syndrome. She was diagnosed with persistent pulmonary hypertension of the newborn, neonatal hypoglycemia and *Bacillus spp.* septicemia.

At 24 hours of life, she developed anemia and marked jaundice. Her physical examination revealed markedly pale, icteric skin and sclera without hepatosplenomegaly and dysmorphic features. Her hemoglobin (Hb) was 7.2 g/dL, MCV 131.5 fL, elevated nucleated red blood cells (nRBCs) count (632 nRBCs per 100 white blood cells) with normal white cells (corrected total white cell count 8.8 $x10^9$/L, neutrophils 52%, lymphocytes 38%) and normal platelet count (160 $x10^9$/L). She also presented hyperbilirubinemia (microbilirubin 19.6mg/dL) so total exchange transfusion and phototherapy were performed.

According to laboratory investigation at the provincial hospital, her and her mother's blood groups were both O-positive. The direct antiglobulin test was negative and G6PD enzyme screening was normal. Extensive investigations for infectious diseases were performed and showed negative for Epstein Barr virus (EBV), cytomegalovirus (CMV), hepatitis B and C virus, parvovirus B19, toxoplasmosis, enterovirus, herpes simplex virus (HSV), syphilis and rubella. High-performance liquid chromatography (HPLC) of hemoglobin revealed no evidence of β-thalassemia or any hemoglobin variant at two months of age and alpha globin gene analysis revealed negative for common alpha globin mutations. No family history was noted of anemia or other hematological disorders. She was admitted at the provincial hospital for two weeks and received multiple blood transfusion before discharge.

A month later, she attended the provincial hospital with symptoms of anemia; her Hb had fallen to 4.8 g/dL so she received 1 unit of blood transfusion and then discharged. At the follow-up clinic, two weeks later, she still presented anemia. Her Hb was 4.7 g/dL and corrected reticulocyte count was 2.6%. For that reason,

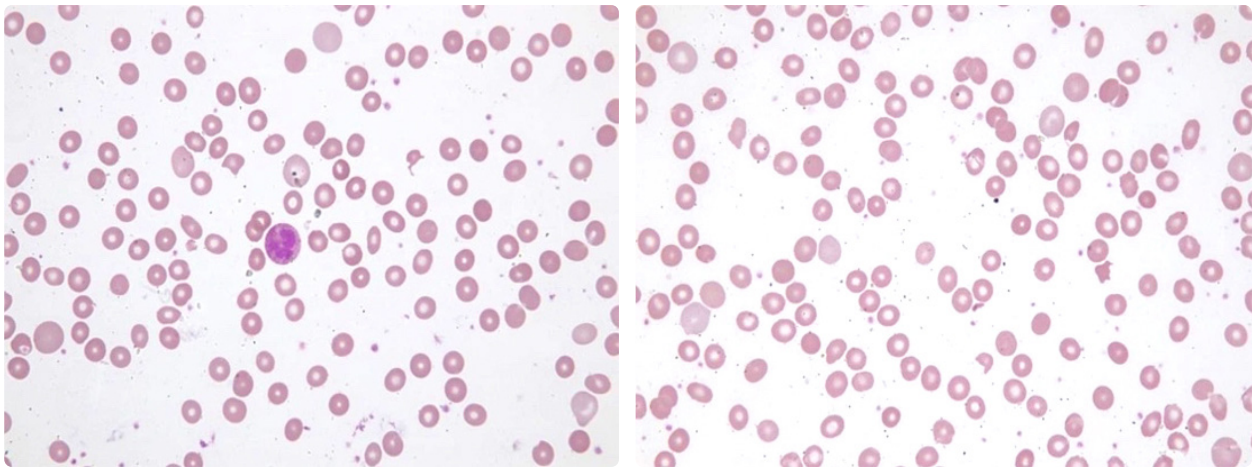

Figure 1 Peripheral blood smear revealed normochromic normocytic red cells, anisopoikilocytosis 1+, few fragmented red cells, polychromasia, and elliptocyte, normal while cells and platelets.

she was referred to the King Chulalongkorn Memorial Hospital (KCMH) due to chronic anemia.

At the KCMH, her physical examination showed body weight 4.5 kg (P10 to 25), height 54 cm (P3 to 10) and head circumference 39 cm (P25), pale conjunctiva, anicteric sclera, no hepatomegaly and her spleen was palpated. Her Hb was 10.2 g/dL after blood transfusion, MCV 80.4 fL, RDW 13.3%, MCHC 32.2 g/dL and corrected reticulocyte count 3.8%. Peripheral blood smear revealed normochromic normocytic red cells, anisopoikilocytosis 1+, few fragmented red cells and elliptocytes and polychromasia and normal while cells and platelets (Figure 1). Paternal completed blood count (CBC) revealed Hb 16.4 g/dL, MCV 83 fL, MCHC 34.6 g/dL, normal white cells, normal platelets and normochromic normocytic red cells in peripheral blood smear. Maternal CBC revealed Hb 12.7 g/dL, MCV 80 fL, MCHC 33.6 g/dL, normal white cells and platelets and normochromic normocytic red cells in peripheral blood smear.

Regarding laboratory evaluation for hemolytic anemia, the eosin-5'-malemide (EMA) binding test was in normal range, specific sequencing for SPTB mutation (spectrin B: Lao PDR, Suandok, Buffalo) was negative and pyruvate kinase level (after blood transfusion for six weeks) was 12.74 IU/g Hb (normal range 11.0-18.9 IU/g Hb). Because clinical presentation was compatible with chronic non-spherocytic hemolytic anemia, trio-whole exome sequencing analysis was performed revealing compound heterozygotes pathogenic variants in the PKLR gene. One missense mutation from her mother was NM_000298.6:c.941T>C,p.Ile314Thr that had been previously reported among Chinese patients with autosomal recessive PKD. The other from her father comprised large deletion 2566 base pairs extending from exons 3 to 9 that was also previously reported among patients with PKD (Figure 2).

Because she was diagnosed as presenting pyruvate kinase deficiency from compound heterozygotes pathogenic variants in the PKLR gene, she has been receiving regular blood transfusion every four weeks and folic supplement because of significant daily symptoms of anemia with baseline Hb <7g/dL. The patient's weight, height, anemic symptoms, pretransfusion Hb level and symptom of cholecystitis were closely observed at every OPD visit.

Discussion

Most glycolytic enzymopathies have an autosomal recessive pattern of inheritance and more than 90% of cases associated with hemolysis are due to pyruvate kinase deficiency (PKD) that was first described in the early 1960s[4]. Heterozygotes of PKD almost always are hematologically normal although their RBCs contain 40% to 60% enzyme activity. Additionally, RBCs of

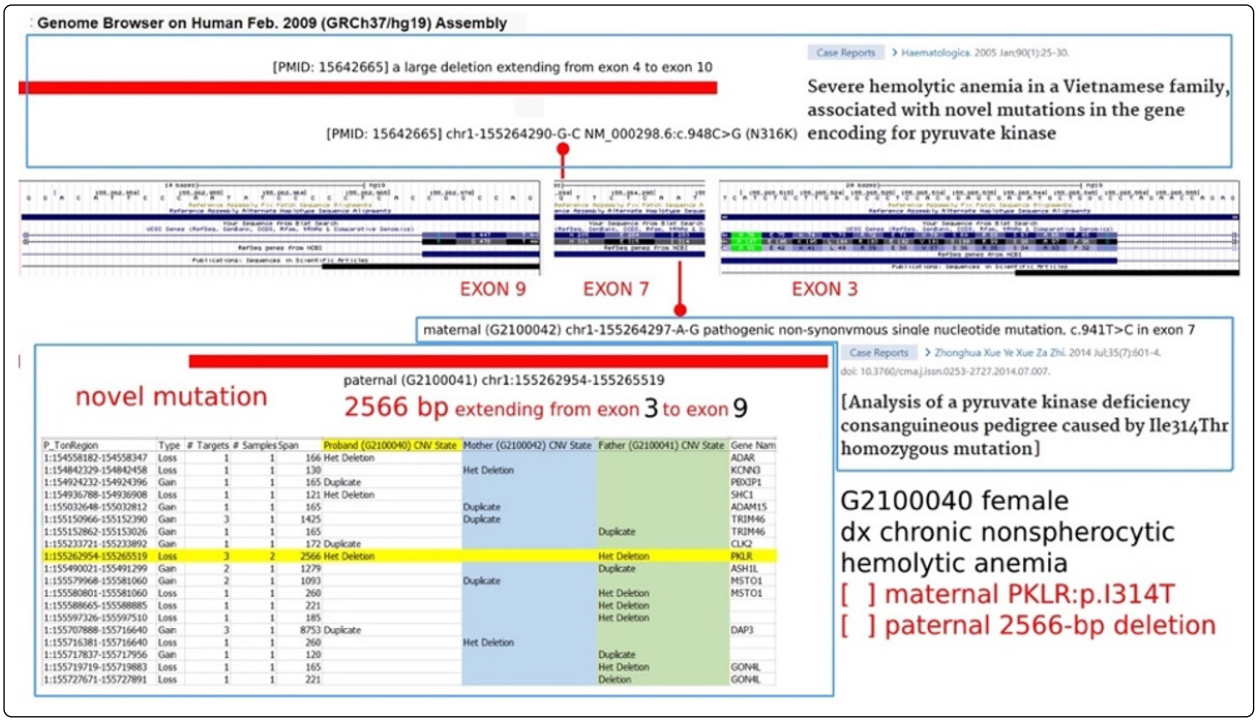

Figure 2 Trio-whole exome sequencing analysis found compound heterozygous pathogenic variants in the PKLR gene, NM_000298.6:c.941T>C,p.Ile314Thr from her mother and large deletion 2566 base pairs extending from exon 3 to exon 9 from her father

homozygotes generally contain 25% residual enzyme activity resulting in mutations in the PKLR gene located on chromosome 1q21 and 300 pathogenic mutations have been described[6]. In contrast to glucose-6-phosphate dehydrogenase (G6PD) deficiency, affecting millions of people, estimated prevalence of PKD among Caucasians is 1 in 20,000 population[1,4,5]. PK enzyme catalyzes phosphoenolpyruvate to pyruvate, resulting in ATP production to maintain the structural and functional integrity of RBCs during their lifespan of 100 to 120 days. When inadequate ATP production occurs due to PKD, RBCs lose their membrane plasticity resulting in cellular dehydration, and are subsequently destroyed in the spleen and liver[4,7-9]. In addition, deficient PK causes the accumulation of glycolytic pathway intermediates such as 2,3-diphosphoglycerate (2,3-DPG) that shifts the hemoglobin-oxygen dissociation curve to the right[10,11]. As a result, PK deficient patients exhibit greater exercise tolerance than the degree of anemia[1,12].

The clinical manifestations of PKD include chronic anemia, reticulocytosis, and indirect hyperbilirubinemia. Anemia in PKD comprises an individually wide spectrum of Hb concentration, most commonly ranging between 6 and 12 g/dL[13]. About 25% of patients experience complications in utero or at the time of birth, including intrauterine growth retardation, hydrops, preterm birth, and perinatal anemia. After birth, most newborns develop severe jaundice and hemolysis requiring phototherapy or simple or exchange transfusions[1].

This patient, at 24 hours of life, developed marked jaundice and her Hb was 7.2 g/dL, elevated nucleated red blood cell count (632 nRBCs per 100 white blood cells) and microbilirubin (MB) 19.6 mg/dL. Total exchange transfusion and phototherapy were performed until hyperbilirubinemia improved. The cause of severe anemia and jaundice in this patient was hemolytic anemia, so the investigations for common causes of severe neonatal jaundice and hemolytic anemia were nondiagnostic including ABO incompatibility, G6PD deficiency, Coombs tests, congenital infection, and peripheral blood smear to demonstrate thalassemia and any red cell membrane defects. She still presents chronic

hemolysis. Also regarding Hb typing (HPLC methods) and alpha gene analysis, the eosin-5'-malemide (EMA) binding test and three common SPTB mutations in hereditary elliptocytosis were normal.

Enzymopathies, such as PKD, should be suspected among patients of all ages with chronic hemolytic anemia in the absence of immune-mediated hemolysis, hemoglobinopathy, or evidence of a red cell membrane disorder[1]. PK level of the patient was determined after blood transfusion for six weeks and reported at 12.74 IU/g Hb (normal range 11.0- to 18.9 IU/g Hb).

Trio-whole exome sequencing analysis was performed revealing compound heterozygous pathogenic variants in the PKLR gene. One missense mutation from her mother was NM_000298.6:c.941T>C,p.Ile314Thr that had been previously reported in a Chinese girl with PKD and homozygous mutation of the PKLR gene, who presented severe chronic nonspherocytic hemolytic anemia with enlarged spleen[14,15]. The other variant, from her father, comprised large deletion 2566 base pairs extending from exon 3 to exon 9 that also had been previously reported in a Vietnamese girl with PKD who presented compound heterozygous pathogenic variants of PK Saigon (N316K) and PK Viet del 4 to 10 experienced from severe transfusion-dependent anemia[16-19].

The PKLR gene, located on chromosome 1q21, consists of 12 exons and encodes for the liver (L) and erythrocyte (R) isoforms of the enzyme according to tissue-specific promoters[20,21]. Ten exons are shared by the two isoforms, while exons 1 and 2 are specifically transcribed to the PK-R and PK-L mRNA, respectively. Several PKD neonates reported severe hepatic disease and developed liver failure, but this patient had transient transaminitis and that spontaneously resolved[22].

Since the second day of life, she has been receiving regular blood transfusion every four weeks and folic supplement because of significant daily symptoms of anemia and baseline Hb < 7 g/dL. After regular transfusion, she not only exhibits no symptoms of anemia but also has gained her proper weight and height. Growth parameters, developments, anemic symptom, pretransfusion Hb and symptom of cholecystitis are closely observed at every OPD visit[4].

From this patient and several case reports, when the frequency of blood transfusion is less than the lifespan of RBCs, it will constitute the major cause of falsely normal PK levels. The molecular testing for PKLR gene mutations plays an important role as a confirmatory test in a patient with suspected PKD but showing normal PK levels and recent blood transfusions. The complications of PKD are closely observed such as aplastic and hemolytic crises from parvovirus B 19 infection, gallbladder disease, bone changes associated with hyperplastic bone marrow, pulmonary hypertension, and thrombosis. Due to an iron overload from regular transfusion and increasing gastrointestinal absorption, annual ferritin monitoring is required[1,4].

Conclusion

A 1-year-old Thai girl with a history of marked jaundice, hemolytic anemia, reticulocytosis and hyperbilirubinemia at 24 hours of life was treated with total exchange transfusion and extensive phototherapy. After that she experienced chronic nonspherocytic hemolytic anemia with an absence of infection, immune-mediated hemolysis, hemoglobinopathy, or evidence of a red cell membrane disorder. Her blood PK level was normal due to recent transfusions. Trio-whole exome sequencing analysis found compound heterozygous pathogenic variants in the PKLR gene, NM_000298.6:c.941T>C,p.Ile314Thr from her mother and large deletion 2566 base pairs extending from exon 3 to exon 9 from her father. Since the second day of life, she has been receiving regular blood transfusions every four weeks and folic supplement because of significant daily symptoms of anemia and baseline Hb < 7 g/dL.

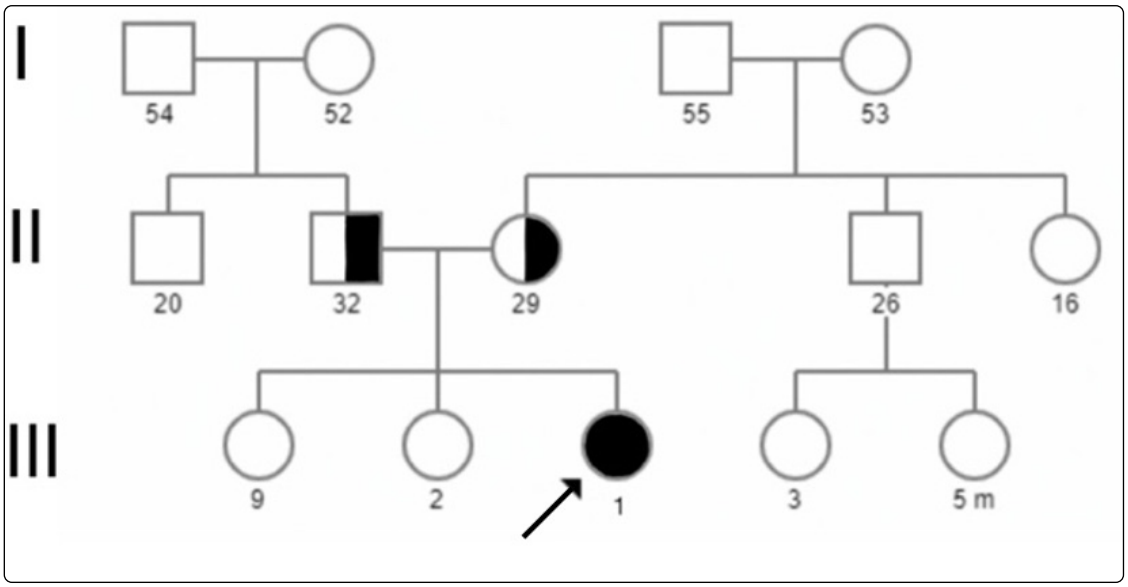

Figure 3 Pedigree of this family, II-2: father with large deletion 2566 base pairs extending from exon 3 to exon 9, II-3: mother with NM_000298.6:c.941T>C,p.Ile314Thr (missense mutation) and III-3: proband with compound heterozygous mutation

References

1. *Grace RF, Glader B. Red blood cell enzyme disorders. Pediatr Clin North Am. 2018;65:579-95.*
2. *Sivashangar A, Gooneratne L, Clark B, Rees D, Jayasinghe S, Laas C. A Sri Lankan girl with a new genetic variant in the PKLR gene causing pyruvate kinase deficiency: a case report. J Med Case Rep. 2021;15:374.*
3. *Grace RF, Zanella A, Neufeld EJ, Morton DH, Eber S, Yaish H, et al. Erythrocyte pyruvate kinase deficiency: 2015 status report. Am J Hematol. 2015;90:825-30.*
4. *Grace RF, Barcellini W. Management of pyruvate kinase deficiency in children and adults. Blood. 2020;136:1241-9.*
5. *Beutler E, Gelbart T. Estimating the prevalence of pyruvate kinase deficiency from the gene frequency in the general white population. Blood. 2000;95:3585-8.*
6. *Bianchi P, Fermo E, Glader B, Kanno H, Agarwal A, Barcellini W, et al. Addressing the diagnostic gaps in pyruvate kinase deficiency: consensus recommendations on the diagnosis of pyruvate kinase deficiency. Am J Hematol. 2019;94:149-61.*
7. *Oski FA, Nathan DG, Sidel VW, Diamond LK. Extreme hemolysis and red-cell distortion in erythrocyte pyruvate kinase deficiency. I. morphology, erythrokinetic and family enzyme studies. N Engl J Med. 1964;270:1023-30.*
8. *Mentzer WC, Jr., Baehner RL, Schmidt-Schönbein H, Robinson SH, Nathan DG. Selective reticulocyte destruction in erythrocyte pyruvate kinase deficiency. J Clin Invest. 1971;50:688-99.*
9. *Nathan DG, Oski FA, Miller DR, Gardner FH. Life-span and organ sequestration of the red cells in pyruvate kinase deficiency. N Engl J Med. 1968;278:73-81.*
10. *Delivoria-Papadopoulos M, Oski FA, Gottlieb AJ. Oxygen-hemoglobulin dissociation curves: effect of inherited enzyme defects of the red cell. Science. 1969;165:601-2.*
11. *Bunn HF, Briehl RW. The interaction of 2,3-diphosphoglycerate with various human hemoglobins. J Clin Invest. 1970;49:1088-95.*
12. *Oski FA, Marshall BE, Cohen PJ, Sugerman HJ, Miller LD. The role of the left-shifted or right-shifted oxygen-hemoglobin equilibrium curve. Ann Intern Med. 1971;74:44-6.*
13. *Tanaka KR, Paglia DE. Pyruvate kinase deficiency. Semin Hematol. 1971;8:367-96.*
14. *He Y, Luo J, Lei Y, Jia S, Liao N. A novel PKLR gene mutation identified using advanced molecular techniques. Pediatr Transplant. 2018;22. doi: 10.1111/petr.13143*
15. *Qu Y, He H, Du J, Hou J, Fu W. Analysis of a pyruvate kinase deficiency consanguineous pedigree caused by Ile314Thr homozygous mutation. Zhonghua Xue Ye Xue Za Zhi. 2014;35:601-4.*
16. *Bianchi P, Fermo E. Molecular heterogeneity of pyruvate kinase deficiency. Haematologica. 2020;105:2218-28.*
17. *Bianchi P, Fermo E, Lezon-Geyda K, van Beers EJ, Morton HD, Barcellini W, et al. Genotype-phenotype correlation and molecular heterogeneity in pyruvate kinase deficiency. Am J Hematol. 2020;95:472-82.*
18. *Fermo E, Bianchi P, Chiarelli LR, Cotton F, Vercellati C, Writzl K, et al. Red cell pyruvate kinase deficiency: 17 new mutations of the PK-LR gene. Br J Haematol. 2005;129:839-46.*
19. *Costa C, Albuisson J, Le TH, Max-Audit I, Dinh KT, Tosi M, et al. Severe hemolytic anemia in a Vietnamese family, associated with novel mutations in the gene encoding for pyruvate kinase. Haematologica. 2005;90:25-30.*

20. *Kanno H, Fujii H, Miwa S. Structural analysis of human pyruvate kinase L-gene and identification of the promoter activity in erythroid cells. Biochem Biophys Res Commun. 1992;188:516-23.*
21. *Noguchi T, Yamada K, Inoue H, Matsuda T, Tanaka T. The L- and R-type isozymes of rat pyruvate kinase are produced from a single gene by use of different promoters. J Biol Chem. 1987;262:14366-71.*
22. *Raphaël MF, Van Wijk R, Schweizer JJ, Schouten-van Meeteren NA, Kindermann A, van Solinge WW, et al. Pyruvate kinase deficiency associated with severe liver dysfunction in the newborn. Am J Hematol. 2007;82:1025-8.*