Pyruvate Kinase Deficiency (PKLR) Sequencing

Red cell pyruvate kinase (PK) deficiency, although relatively rare, is the most common glycolytic defect resulting in congenital nonspherocytic hemolytic anemia (CNSHA). The PKLR gene produces PK in the liver and red blood cells (RBCs) that converts phosphoenolpyruvate to pyruvate, creating 50% of the red cell adenosine triphosphate (ATP). Pathogenic variants in PKLR cause reduced PK function, leading to the accumulation of intermediate glycolysis by-products and a shortage of ATP in RBCs. This results in shortened RBC lifespan and damaged cells are removed from circulation by the spleen. Clinical features of PK deficiency are highly variable, ranging from well-compensated anemia to severe disease with lifelong transfusion dependency. Other clinical manifestations may include jaundice, gallstones, iron overload, and potential for other complications.

Typical testing strategy includes PK activity level followed by molecular testing to confirm diagnosis in individuals with reduced PK activity and/or clinical findings. Molecular testing is the most reliable method of identifying heterozygous PKLR variant carriers. Carriers often have intermediate levels of PK activity, but are not at risk for clinical symptoms.

DISEASE OVERVIEW

Prevalence
Varies by ethnicity; 1 in 20,000 Caucasians, higher prevalence in Pennsylvania Amish and Romani.

Clinical Findings
Preterm labor/prematurity
Prenatal growth restriction
Prenatal hydrops
Indirect hyperbilirubinemia/jaundice
  • Most newborns are treated with phototherapy; many require exchange transfusion
Chronic hemolytic anemia of varying severity
  • Infants and young children may be transfusion-dependent prior to splenectomy
  • Anemia may stabilize in adulthood; however, exacerbations can result with infections, pregnancy, or stress
  • 2,3 diphosphoglycerate is elevated and shifts oxygen dissociation curve to favor unloading of oxygen in tissues, thus, anemia may be better tolerated than in other conditions
Reticulocytosis
  • Increase may not be proportional to severity of anemia
Reduced red cell PK activity
  • Contamination with normal donor RBCs in transfused patients or compensatory persistence of the M2 fetal isoform may occasionally result in normal PK activity
Clinical complications
  • Iron overload
  • Gallstones
  • Less common: aplastic crises, osteopenia/bone fragility, extramedullary hematopoiesis, postsplenectomy sepsis, pulmonary hypertension, or leg ulcers

Surgical Treatments
Splenectomy
  • Splenectomy may moderately improve anemia and reduce transfusion burden
Cholecystectomy

GENETICS

Gene
PKLR

Inheritance
Autosomal recessive

Test Methodology
PKLR sequencing: polymerase chain reaction (PCR) followed by bidirectional sequencing of all coding regions and intron-exon boundaries, 5’ untranslated region, and deep intronic variants c.1269+43T>C and c.1269+44C>T (also known as IVS9+43T>C and IVS9+44C>T, respectively)
Variants

Over 250 disease-associated \textit{PKLR} variants have been described:
- c.1529G>A: common variant in U.S. and Europe
- c.1456C>T: common variant in Southern Europe, homozygosity associated with mild phenotype
- c.1468C>T: common variant in Asia
- c.1436G>A: Pennsylvania Amish founder variant
- 1,149 bp deletion: Romani founder variant known as “PK Gypsy” (not detectable by sequencing alone)

Genotype-Phenotype Associations

PK enzyme activity is not correlated with genotype.

Individuals with two causative missense variants have lower likelihood of splenectomy, fewer lifetime transfusions, and lower rate of iron overload versus individuals with nonmissense variants (ie, frameshift, nonsense, indels, large deletions, or splicing variants).

TEST INTERPRETATION

Sensitivity/Specificity
- Clinical sensitivity: 98%²
- Analytical sensitivity/specificity: 99%

Results

Two pathogenic \textit{PKLR} variants on opposite chromosomes
- Consistent with a diagnosis of PK deficiency

One pathogenic \textit{PKLR} variant identified
- At least a carrier of PK deficiency, may be affected if a second unidentified variant is present on opposite chromosome

No pathogenic variants identified
- Significantly reduces the likelihood of PK deficiency or carrier status

\textit{PKLR} sequencing may identify variants of unknown clinical significance

Limitations of Sanger Sequencing

Not detected:
- Large deletions/duplications, including the Romani founder variant
- Repeat element insertions
- Deep intronic variants other than those targeted
- Regulatory region variants outside of the 5'UTR

Diagnostic errors can occur due to rare sequence variation

REFERENCES

