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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.

Tests to Consider

Pyruvate Kinase Deficiency (PKLR) Sequencing 3002059

Method: Polymerase Chain Reaction/Sequencing

- Use to confirm suspected PK deficiency in individuals with abnormal PK enzyme activity and/or clinical findings.
- Use to assess carrier status for PK deficiency.

Pyruvate Kinase 0080290

Method: Quantitative Enzymatic

Preferred initial screening test to assess for PK deficiency.

Familial Mutation, Targeted Sequencing 2001961

Method: Polymerase Chain Reaction/Sequencing

Use to test for a known familial *PKLR* variant previously identified in a family member.

Hereditary Hemolytic Anemia Panel Sequencing 2012052

Method: Massively Parallel Sequencing

- Use to determine etiology of unexplained hemolytic anemia or family history of unexplained hemolytic anemia.
- Use to determine etiology of unexplained hyperbilirubinemia in neonates.

Disease Overview

Prevalence

Varies by ethnicity; 1 in 20,000 White individuals, higher prevalence in Pennsylvania Amish and Romani individuals[1]

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 s*equencing: 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 *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%
- Analytical sensitivity/specificity: 99%

Results

Two pathogenic *PKLR* variants on opposite chromosomes:

- Consistent with a diagnosis of PK deficiency

One pathogenic *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

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

1. Beutler E, Gelbart T. Estimating the prevalence of pyruvate kinase deficiency from the gene frequency in the general white population. Blood. 2000;95(11):3585-3588. PubMed

Additional Resources

Canu G, De Bonis M, Minucci A, et al. Red blood cell PK deficiency: An update of PK-LR gene mutation database. Blood Cells Mol Dis. 2016;57:100-109. PubMed

Grace RF, Bianchi P, van Beers EJ, et al. Clinical spectrum of pyruvate kinase deficiency: data from the Pyruvate Kinase Deficiency Natural History Study. Blood. 2018;131(20):2183-2192. PubMed

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Content Review August 2019 | Last Update April 2021