Hereditary Hemolytic Anemia Sequencing, 28 Genes

Indications for Ordering

Determine etiology, elicit inheritance pattern, and assess recurrence risk in individuals with
- Unexplained hemolytic anemia
- Unexplained hyperbilirubinemia in neonates
- Family history of unexplained hemolytic anemia
- Pregnancy with hydrops fetalis of unknown etiology

Test Description

- Targeted capture of all coding regions and intron/exon boundaries followed by massively parallel sequencing
- Sanger sequencing
  - Provides data for bases with insufficient coverage by massively parallel sequencing

Tests to Consider

Typical testing strategy
See ARUP Consult’s Hemolytic Anemias Testing algorithm

Primary test
Hereditary Hemolytic Anemia Sequencing, 28 Genes 2012052
- Confirm etiology of hemolytic anemia in individuals with hemolysis or a family history of hemolytic anemia

Related tests
Osmotic Fragility, Erythrocyte 2002257
- Functional testing of red blood cell (RBC) sensitivity to osmotic stress
- Do not use to distinguish between spherocytes in hereditary spherocytosis and acquired autoimmune hemolytic anemia

RBC Band 3 Protein Reduction in Hereditary Spherocytosis 2008460
- Use to confirm diagnosis of hereditary spherocytosis when hemolytic anemia and spherocytes are present

Glucose-6-Phosphate Dehydrogenase 0080135
- Preferred initial screening test for G6PD deficiency

Glucose-6-Phosphate Dehydrogenase Deficiency (G6PD) Sequencing 2007163
- Preferred test for individuals of high-risk ethnic backgrounds other than those of African descent
- Detects most G6PD deficiency-causing gene variants

Glucose-6-Phosphate Dehydrogenase (G6PD) 2 Mutations 0051684
- Preferred genetic test for individuals of African descent
- Detects the single most common pathogenic G6PD variant (A- allele) in individuals of African descent

Pyruvate Kinase 0080290
- Preferred initial test to screen for pyruvate kinase deficiency

Hemoglobin Evaluation Reflexive Cascade 2005792
- Optimal test for the initial and confirmatory diagnosis of any suspected hemoglobinopathy

Disease Overview

Prevalence
Hereditary hemolytic anemia (HHA) disorders – 1/500-1,100

Symptoms
Highly variable clinical presentation
- Anemia
- Fatigue
- Gallstones
- Hyperbilirubinemia/jaundice
- Pallor
- Scleral icterus
- Splenomegaly

Physiology
HHA encompasses a diverse group of heterogeneous disorders
- Characterized by premature RBC destruction and anemia due to intrinsic RBC defects

RBC membrane disorders
- RBC membrane weakens, resulting in irregular shape, increased fragility, and hemolysis
- Most common types of RBC membrane disorders
  - Hereditary spherocytosis
  - Hereditary elliptocytosis or pyropoikilocytosis
  - Dehydrated hereditary stomatocytosis (xerocytosis)
Hereditary spherocytosis (HS)
• Inheritance
  o ~75% autosomal dominant
  o ~25% autosomal recessive or de novo
• Most common RBC membrane disorder
  o 1/2,000 northern Europeans
• Characterized by spherically shaped RBCs with decreased deformability
• RBC membrane proteins
  o Ankyrin
  o Band 3
  o α-spectrin
  o β-spectrin
  o Protein 4.2

Hereditary elliptocytosis or pyropoikilocytosis (HE/HPP)
• Inheritance
  o HE – autosomal dominant
  o HPP – autosomal recessive
• Prevalence of HE – 1/2,000-4,000 worldwide
  o Higher in individuals of African or Mediterranean descent
• Characterized by elliptically shaped RBCs on peripheral blood smear
• Defects in RBC membrane proteins α- and β-spectrin and less commonly protein 4.1
• Patients with HE are generally asymptomatic
• 10% have moderate to severe anemia

Dehydrated hereditary stomatocytosis (xerocytosis)
• Inheritance
  o Autosomal dominant
  o Autosomal recessive
• Characterized by
  o Decreased intracellular potassium content
  o Loss of cell water
  o Increased cytoplasmic viscosity
  o Increased mean cell hemoglobin concentration
• PIEZO1 is most commonly involved gene

RBC enzymopathies
• Inheritance
  o Most disorders are autosomal recessive
  o X-linked forms
    ▪ G6PD deficiency
    ▪ Phosphoglycerate kinase 1 (PGK1) deficiency
• Deficiencies of enzymes involved with
  o Glycolysis
  o Hexose monophosphate shunt
  o Glutathione metabolism
  o Nucleotide metabolism
• >20 disorders recognized
  o Common RBC enzymopathies
    ▪ G6PD deficiency
    ▪ Pyruvate kinase deficiency
  o Usually associated with normocytic normochromic hemolytic anemia with no specific abnormalities of RBC morphology
  o Severity of hemolysis is variable
    ▪ May be a result of an external stressor (eg, infection, administration of drugs, or ingestion of some foods)
    ▪ Nonhematological manifestations possible (eg, myopathy, neurological dysfunction, intellectual disability)
  o Diagnosis based on detection of reduced specific enzyme activity and/or molecular testing to identify causative variant(s)

Hemoglobinopathies
• Quantitative defect in biosynthesis of one type of hemoglobin (Hb) chain or a structurally abnormal Hb
  o Alpha or beta thalassemia
    ▪ Quantitative defect in the synthesis of either the alpha- or beta-globin chain
    ▪ Unpaired subunits precipitate, bind to the RBC membrane, and lead to hemolysis
  o Structural Hb variants
    ▪ Result from a structurally abnormal Hb that may polymerize, precipitate, or crystalize within the RBC
    ▪ Leads to membrane changes and hemolysis
    ▪ Sickle cell diseases
    ▪ Unstable Hb variants

Diagnostic issues
Molecular testing for hemolytic anemia is indicated when initial test results do not explain
• Clinical outcome
• Mode of inheritance

Treatment issues
Splenectomy should be avoided in patients with some forms of hereditary stomatocytosis
• May predispose patient to life-threatening thrombotic events

Genetics
Genes tested – see table

Test Interpretation
Clinical sensitivity – unknown
## Results

- **Positive**
  - One or more pathogenic variants detected
    - Autosomal dominant genes
    - One pathogenic variant predicts an HHA disorder
    - Autosomal recessive genes
    - One pathogenic variant predicts carrier status for an HHA disorder
    - Two pathogenic variants on opposite chromosomes predicts an HHA disorder
    - X-linked genes
    - In females, one pathogenic variant predicts carrier status
    - In males, one pathogenic variant predicts an HHA disorder

- **Negative**
  - No pathogenic variants associated with an HHA disorder were identified
    - Does not exclude a diagnosis of HHA

- **Inconclusive**
  - Variants of unknown clinical significance were identified

## Limitations

- **Not detected**
  - Variants in genes not tested
    - Alpha- and beta-globin genes are not analyzed due to high level of gene homology and frequency of large deletions
  - Large exonic deletions/duplications
  - Deep intronic or regulatory region variants
  - Small deletions or insertions may not be detected
  - Diagnostic errors can occur due to rare sequence variation
  - Presence of a highly homologous pseudogene may interfere with variant detection in *PGK1*

## References


### Gene Symbol | Gene Description | NM # | OMIM # | Inh.* | Associated Disorders | Other Associated Symptoms
--- | --- | --- | --- | --- | --- | ---
ADA | Adenosine deaminase | 000022 | 102730 | AD | Elevated adenosine deaminase | 
AK1 | Adenylate kinase 1 | 000476 | 103000 | AR | Adenylate kinase deficiency | 
ALDOA | Aldolase A | 000334 | 103850 | AR | ALDOA deficiency (glycogen storage disease XII) | Exertional myopathy, muscle weakness
ANK1 | Ankyrin 1 | 000037 | 107008 | AD/AR | Spherocytosis type 1 | 
CYB5R3 | Cytochrome b5 reductase 3 (DIA1) | 000398 | 613213 | AR | Methemoglobinemia type I | Hypoxia, cyanosis, hypoxia
EPB41 | Erythrocyte membrane protein band 4.1 | 004437 | 130500 | AD/AR | Elliptocytosis 1, pyropoikilocytosis | 
EPB42 | Erythrocyte membrane protein band 4.2 | 000119 | 177070 | AR | Spherocytosis type 5 | 
G6PD | Glucose-6-phosphate dehydrogenase | 001042351 | 305900 | XL | Glucose-6-phosphate dehydrogenase deficiency | 
GCLC | Glutamate-cysteine ligase, catalytic subunit | 001498 | 606857 | AR | Glutamate-cysteine ligase deficiency | 
GPI | Glucose phosphate isomerase | 000175 | 172400 | AR | Glucose phosphate isomerase deficiency | With or without neurologic deficits
GSR | Glutathione reductase | 000637 | 138300 | AR | Glutathione reductase deficiency | 
GSS | Glutathione synthetase | 000178 | 601002 | AR | Glutathione synthetase deficiency | 5-oxoprolinuria, metabolic acidosis, neurological dysfunction
HK1 | Hexokinase 1 | 000188 | 142600 | AR | Hexokinase deficiency | 
NT5C3A | S’ nucleotidase, cytosolic IIIA | 016489 | 606224 | AR | Pyrimidine 5’ nucleotidase deficiency (UMPH1 deficiency) | 
PFKL | Phosphofructokinase, liver | 002626 | 171860 | AR | Phosphofructokinase deficiency | 
PFKM | Phosphofructokinase, muscle | 000289 | 610681 | AR | Muscle phosphofructokinase deficiency (glycogen storage disease VII) | Exertional myopathy

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<table>
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<tr>
<th>Gene Symbol</th>
<th>Gene Description</th>
<th>NM #</th>
<th>OMIM #</th>
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<th>Associated Disorders</th>
<th>Other Associated Symptoms</th>
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<td>Phosphoglycerate kinase 1</td>
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* Inh = inheritance; AR = autosomal recessive; AD = autosomal dominant; XL = X-linked