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® 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 (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
  - ~75% autosomal dominant
  - ~25% autosomal recessive or de novo
- Most common RBC membrane disorder
  - 1/2,000 northern Europeans
- Characterized by spherically shaped RBCs with decreased deformability
- RBC membrane proteins
  - Ankyrin
  - Band 3
  - α-spectrin
  - β-spectrin
  - Protein 4.2

Hereditary elliptocytosis or pyropoikilocytosis (HE/HPP)
- Inheritance
  - HE – autosomal dominant
  - HPP – autosomal recessive
- Prevalence of HE – 1/2,000-4,000 worldwide
  - 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
  - Autosomal dominant
- Characterized by
  - Decreased intracellular potassium content
  - Loss of cell water
  - Increased cytoplasmic viscosity
  - Increased mean cell hemoglobin concentration
- PIEZO1 is most commonly involved gene

RBC enzymopathies
- Inheritance
  - Most disorders are autosomal recessive
- X-linked forms
  - G6PD deficiency
  - Phosphoglycerate kinase 1 (PGK1) deficiency
- Deficiencies of enzymes involved with
  - Glycolysis
  - Hexose monophosphate shunt
  - Glutathione metabolism
  - Nucleotide metabolism

• >20 disorders recognized
  - Common RBC enzymopathies
    - G6PD deficiency
    - Pyruvate kinase deficiency
  - Usually associated with normocytic normochromic hemolytic anemia with no specific abnormalities of RBC morphology
  - Severity of hemolysis is variable
    - May be a result of an external stressor (e.g., infection, administration of drugs, or ingestion of some foods)
    - Nonhematological manifestations possible (e.g., myopathy, neurological dysfunction, intellectual disability)
    - 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
- 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
- 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


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