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 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
  - ~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 (eg, infection, administration of drugs, or ingestion of some foods)
  - Nonhematological manifestations possible (eg, 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

- Bolton-Maggs PH, Langer JC, et al. Guidelines for the diagnosis and management of hereditary spherocytosis – 2011 update. Br J Haematol. 2012;156(1):37-49
- Gallagher PG. Abnormalities of the erythrocyte membrane. Pediatr Clin North Am. 2013;60(6):1349-1362
- Koralkova P, van Solinge WW, et al. Rare hereditary red blood cell enzymopathies associated with hemolytic anemia- pathophysiology, clinical aspects, and laboratory diagnosis. Int Jnl Lab Hem. 2014;36:388-397

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	000034	103850	AR	ALDOA deficiency (glycogen storage disease XII)	Exertional myopathy, muscle weakness
ANK1	Ankyrin 1	000037	607008	AD/AR	Spherocytosis type 1	
CYB5R3	Cytochrome b5 reductase 3 (DIA1)	000398	613213	AR	Methemoglobinemia type I	Hypoxia, cyanosis hypoxia
					Methemoglobinemia type II	Hypoxia, cyanosis, intellectual disability, microcephaly, neurologic symptoms
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	5’ 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

Gene Symbol	Gene Description	NM #	OMIM #	Inh.*	Associated Disorders	Other Associated Symptoms
PGK1	Phosphoglycerate kinase 1	000291	311800	XL	Phosphoglycerate kinase deficiency	Myopathy, neurological dysfunction
PIEZO1	Piezo-type mechanosensitive ion channel component 1	001142864	611184	AD	Dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal edema (hereditary xerocytosis)	
PKLR	Pyruvate kinase (liver and red blood cell)	000298	609712	AR	Pyruvate kinase deficiency	
SLC4A1	Solute carrier family 4, anion exchanger, member 1 (erythrocyte membrane protein band 3, AE1)	000342	109270	AD/AR	Spherocytosis type 4, elliptocytosis type 4, stomatocytosis, acanthocytosis, distal renal tubular acidosis with hemolytic anemia	
SLCO1B1	Solute carrier organic anion transporter family, member 1B1	006446	604843	AR (digenic)	Rotor type hyperbilirubinemia	
SLCO1B3	Solute carrier organic anion transporter family, member 1B3	019844	605495	AR (digenic)	Rotor type hyperbilirubinemia	
SPTA1	Spectrin alpha	003126	182860	AD/AR	Elliptocytosis 2, spherocytosis type 3, pyropoikilocytosis, elliptopoikilocytosis	
SPTB	Spectrin beta	000347	182870	AD/AR	Elliptocytosis 3, spherocytosis type 2, neonatal hemolytic anemia	
TPI1	Triosephosphate isomerase 1	000365	190450	AR	Triosephosphate isomerase deficiency	Myopathy
UGT1A1	UDP glycosyltransferase 1 family, polypeptide A1	000463	191740	AR	Crigler-Najjar syndrome type I	Kernicterus, neurologic dysfunction
					Crigler-Najjar syndrome type II, hyperbilirubinemia (unconjugated), Gilbert syndrome	
UGT1A6	UDP glycosyltransferase 1 family, polypeptide A6	001072	606431	AR	UGT1A6 deficiency	
UGT1A7	UDP glycosyltransferase 1 family, polypeptide A7	019077	606432	AR	UGT1A7 deficiency	

* Inh = inheritance; AR = autosomal recessive; AD = autosomal dominant; XL = X-linked