

Hereditary Hemolytic Anemia Panel, Sequencing

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 Panel Sequencing 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 crystallize 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
<i>ADA</i>	Adenosine deaminase	000022	102730	AD	Elevated adenosine deaminase	
<i>AK1</i>	Adenylate kinase 1	000476	103000	AR	Adenylate kinase deficiency	
<i>ALDOA</i>	Aldolase A	000034	103850	AR	ALDOA deficiency (glycogen storage disease XII)	Exertional myopathy, muscle weakness
<i>ANK1</i>	Ankyrin 1	000037	607008	AD/AR	Spherocytosis type 1	
<i>CYB5R3</i>	Cytochrome b5 reductase 3 (DIA1)	000398	613213	AR	Methemoglobinemia type I	Hypoxia, cyanosis hypoxia
					Methemoglobinemia type II	Hypoxia, cyanosis, intellectual disability, microcephaly, neurologic symptoms
<i>EPB41</i>	Erythrocyte membrane protein band 4.1	004437	130500	AD/AR	Elliptocytosis 1, pyropoikilocytosis	
<i>EPB42</i>	Erythrocyte membrane protein band 4.2	000119	177070	AR	Spherocytosis type 5	
<i>G6PD</i>	Glucose-6-phosphate dehydrogenase	001042351	305900	XL	Glucose-6-phosphate dehydrogenase deficiency	
<i>GCLC</i>	Glutamate-cysteine ligase, catalytic subunit	001498	606857	AR	Glutamate-cysteine ligase deficiency	
<i>GPI</i>	Glucose phosphate isomerase	000175	172400	AR	Glucose phosphate isomerase deficiency	With or without neurologic deficits
<i>GSR</i>	Glutathione reductase	000637	138300	AR	Glutathione reductase deficiency	
<i>GSS</i>	Glutathione synthetase	000178	601002	AR	Glutathione synthetase deficiency	5-oxoprolinuria, metabolic acidosis, neurological dysfunction
<i>HK1</i>	Hexokinase 1	000188	142600	AR	Hexokinase deficiency	
<i>NT5C3A</i>	5' nucleotidase, cytosolic IIIA	016489	606224	AR	Pyrimidine 5' nucleotidase deficiency (UMPH1 deficiency)	
<i>PFKL</i>	Phosphofructokinase, liver	002626	171860	AR	Phosphofructokinase deficiency	
<i>PFKM</i>	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
<i>PGK1</i>	Phosphoglycerate kinase 1	000291	311800	XL	Phosphoglycerate kinase deficiency	Myopathy, neurological dysfunction
<i>PIEZO1</i>	Piezo-type mechanosensitive ion channel component 1	001142864	611184	AD	Dehydrated hereditary stomatocytosis with or without pseudohyperkalemia and/or perinatal edema (hereditary xerocytosis)	
<i>PKLR</i>	Pyruvate kinase (liver and red blood cell)	000298	609712	AR	Pyruvate kinase deficiency	
<i>SLC4A1</i>	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	
<i>SLCO1B1</i>	Solute carrier organic anion transporter family, member 1B1	006446	604843	AR (digenic)	Rotor type hyperbilirubinemia	
<i>SLCO1B3</i>	Solute carrier organic anion transporter family, member 1B3	019844	605495	AR (digenic)	Rotor type hyperbilirubinemia	
<i>SPTA1</i>	Spectrin alpha	003126	182860	AD/AR	Elliptocytosis 2, spherocytosis type 3, pyropoikilocytosis, elliptopoikilocytosis	
<i>SPTB</i>	Spectrin beta	000347	182870	AD/AR	Elliptocytosis 3, spherocytosis type 2, neonatal hemolytic anemia	
<i>TPI1</i>	Triosephosphate isomerase 1	000365	190450	AR	Triosephosphate isomerase deficiency	Myopathy
<i>UGT1A1</i>	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	
<i>UGT1A6</i>	UDP glycosyltransferase 1 family, polypeptide A6	001072	606431	AR	UGT1A6 deficiency	
<i>UGT1A7</i>	UDP glycosyltransferase 1 family, polypeptide A7	019077	606432	AR	UGT1A7 deficiency	

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