Alpha (α) thalassemia is the most common inherited disorder of hemoglobin (Hb) worldwide and is caused by HBA1 and HBA2 gene variants. Decreased or absent synthesis of the hemoglobin (Hb) α chain results in clinical presentations ranging from asymptomatic silent carriers to severe anemia and fetal lethality. The two clinically significant forms of α thalassemia are Hb Bart hydrops fetalis syndrome and hemoglobin H (HbH) disease. Alpha thalassemia is found more often in certain ethnicities, including African, African American, Mediterranean, Middle Eastern, and Southeast Asian.

Disease Overview

Prevalence and/or Incidence

• Most common inherited disorder of Hb worldwide
• Carrier frequencies in high-risk populations:
  • African, African American: 1/3
  • Middle Eastern, Southeast Asian: 1/20
  • Mediterranean: 1/30-50
• Hb Bart hydrops fetalis syndrome and HbH disease are more frequent in Southeast Asian, Asian Indian, and Mediterranean populations than in African populations.

Symptoms

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Associated Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>α Thalassemia silent carrier</td>
<td>Typically asymptomatic, though borderline anemia or mild microcytosis may be present</td>
</tr>
<tr>
<td></td>
<td>Often misdiagnosed as iron deficiency</td>
</tr>
<tr>
<td></td>
<td>Normal Hb electrophoresis</td>
</tr>
<tr>
<td>α Thalassemia trait</td>
<td>Mild microcytic anemia may be present</td>
</tr>
<tr>
<td></td>
<td>Often misdiagnosed as iron deficiency</td>
</tr>
<tr>
<td></td>
<td>Normal Hb electrophoresis</td>
</tr>
<tr>
<td>HbH disease</td>
<td>Moderate to severe form of α thalassemia</td>
</tr>
<tr>
<td></td>
<td>Moderate microcytic hypochromic anemia</td>
</tr>
<tr>
<td></td>
<td>Hemolysis with Heinz bodies</td>
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<tr>
<td></td>
<td>Splenomegaly</td>
</tr>
<tr>
<td></td>
<td>Rare extramedullary hematopoiesis</td>
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<tr>
<td></td>
<td>Propensity for acute hemolysis after oxidative stress, drug therapy, or infection</td>
</tr>
</tbody>
</table>

Tests to Consider

Alpha Thalassemia (HBA1 and HBA2) Deletion/Duplication with reflex to Hb Constant Spring 3003651
Method: Multiplex Ligation-dependent Probe Amplification with Sanger sequencing confirmation of HbCS

• Preferred first-tier genetic test for confirmation of suspected α thalassemia or α thalassemia trait
• Detects common, rare, and novel deletions or duplications in the α-globin gene cluster and its HS-40 regulatory region
• Multiplex ligation-dependent probe amplification (MLPA) used to detect Hb Constant Spring (HbCS) (HBA2 c.427T>C; p.Ter143Gln); targeted Sanger sequencing is performed to assess HbCS copy number in absence of a concurrent HBA2 deletion.

Alpha Globin (HBA1 and HBA2) Deletion/Duplication 2011622
Method: Multiplex Ligation-dependent Probe Amplification

• First-tier genetic test for confirmation of suspected α thalassemia or α thalassemia trait
• Detects common, rare, and novel deletions or duplications in the α-globin gene cluster (HBZ, HBM, HBA1, HBA2, HBQ1) and its HS-40 regulatory region

Alpha Globin (HBA1 and HBA2) Sequencing and Deletion/Duplication 2011708
Method: Polymerase Chain Reaction/Sequencing/Multiplex Ligation-dependent Probe Amplification.

• Comprehensive genetic test for detection of α thalassemia or α thalassemia trait
• Detect deletional and nondeletional variants in HBA1 and HBA2

Alpha Thalassemia (HBA1 and HBA2) Deletion/Duplication with reflex to Hb Constant Spring, Fetal 3003656
Method: Multiplex Ligation-dependent Probe Amplification with Sanger sequencing confirmation of HbCS

• Diagnostic testing for α thalassemia in fetus with suggestive clinical findings or at risk for α thalassemia due familial HBA1/HBA2 deletions or HbCS variant
• Detects common, rare, and novel deletions or duplications in the α-globin gene cluster and its HS-40 regulatory region
• MLPA is used to detect HbCs (HBA2 c.427T>C; p.Ter143Gln); targeted Sanger sequencing is performed to assess HbCS copy number.
Pathophysiology

Typically, individuals have four functioning α-globin genes (αα/αα). Two genes, HBA1 and HBA2, are present on each copy of chromosome 16 and α-globin chains function as subunits of fetal Hb (HbF: α2γ2) and adult Hb (HbA: α2β2). The number of α-globin genes deleted or inactivated correlates with different α-thalassemia phenotypes. Genotype/phenotype correlations in α-thalassemia are complex and may be influenced by coinheritance of other Hb variants or α-globin gene duplications.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α Thalassemia silent carrier</td>
<td>-α/αα</td>
</tr>
<tr>
<td>α Thalassemia trait</td>
<td>-α/-α</td>
</tr>
<tr>
<td></td>
<td>-/-αα</td>
</tr>
<tr>
<td>HbH disease</td>
<td>-/-α</td>
</tr>
<tr>
<td>Hb Bart hydrops fetalis syndrome</td>
<td>--/--</td>
</tr>
</tbody>
</table>

Genetics

Genes

HBA1 and HBA2

Inheritance

Autosomal recessive

Variants

- HBA1 and HBA2 large gene deletions account for approximately 90% of pathogenic α-thalassemia variants.
  - -α3.7 and -α4.2 deletions result in the deletion of a single gene.
  - -(α)20.5, -SEA, -MED-I, -FIL, and -THAI deletions result in the deletion of both HBA1 and HBA2 genes from the same chromosome
- Sequence variants and regulatory region variants occur mainly in HBA2 and account for up to 15% of causative variants.
  - Nondeletional variants include:
    - Sequence variants that inactivate the gene
    - Small insertions/deletions
    - Variants that result in unstable α-globin protein (eg, Hb Constant Spring or HbC-S)
  - Nondeletional α-globin variants may be pathogenic or benign.
    - Both may result in an abnormal protein detectable by Hb evaluation.
    - Pathogenic nondeletional variants often have a more severe effect than single gene deletions.
Alpha-globin gene duplication results in three or more active α-globin genes on a single chromosome.

- Typically benign
- May alter expected clinical phenotypes and hematological features when coinherited with beta (β) thalassemia

Test Interpretation

Alpha Globin (HBA1 and HBA2) Sequencing and Deletion/Duplication

Sensitivity/Specificity

- Analytical sensitivity/specificity: 99% for both duplication/deletion analysis and sequencing
- Clinical sensitivity: most pathogenic HBA1 and/or HBA2 gene variants are large deletions not detectable by sequencing
  - Deletion: at least 90%, varies by ethnicity
  - Sequencing: up to 15%, varies by ethnicity

Results

<table>
<thead>
<tr>
<th>Result</th>
<th>Variant(s) Detected</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Genotype (-a/aa)</td>
<td>Silent carrier</td>
</tr>
<tr>
<td></td>
<td>Genotype (-a/-a) or (--/aa)</td>
<td>α Thalassemia trait</td>
</tr>
<tr>
<td></td>
<td>1 pathogenic sequence variant</td>
<td>Silent carrier or α thalassemia trait</td>
</tr>
<tr>
<td></td>
<td>Genotype (-a/aa)</td>
<td>HbH disease</td>
</tr>
<tr>
<td></td>
<td>Genotype (--/--)</td>
<td>Hb Bart hydrops fetalis syndrome</td>
</tr>
<tr>
<td></td>
<td>Genotype (aa/aaa)</td>
<td>Extra functional alpha globin gene copy is present</td>
</tr>
<tr>
<td>Negative</td>
<td>No pathogenic variants detected</td>
<td>Greatly decreased probability that the individual is affected with, or a carrier of, α thalassemia</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>Variant(s) of uncertain clinical significance identified</td>
<td>Unknown clinical significance</td>
</tr>
</tbody>
</table>

Limitations

- Diagnostic errors can occur due to rare sequence variations.
- Sequence analysis will not detect all regulatory region variants or variants in α-globin cluster genes other than HBA1 and HBA2.
- Sequencing of both HBA1 and HBA2 may not be possible in individuals harboring large α-globin deletions on both alleles.
- This assay is unable to sequence HBA2-HBA1 fusion genes; thus, HBA1 or HBA2 sequence variants occurring in cis with a 3.7 kb deletion or other HBA2-HBA1 hybrid gene will not be detected.
- It may not be possible to determine the phase of identified sequence variants.
- Specific breakpoints of large deletions/duplications will not be determined; therefore, it may not be possible to distinguish deletion variants of similar size.
- Individuals carrying both a deletion and duplication within the α-globin gene cluster may appear to have a normal number of α-globin gene copies.
- Rare syndromic or acquired forms of α-thalassemia associated with ATRX variants will not be detected.

References

Related Tests

Hemoglobin Evaluation with Reflex to Electrophoresis and/or RBC Solubility 0050610

Method: High Performance Liquid Chromatography/Electrophoresis/RBC Solubility

Hemoglobin Evaluation Reflexive Cascade 2005792

Method: High Performance Liquid Chromatography/Electrophoresis/RBC Solubility/Polymerase Chain Reaction/Fluorescence Resonance Energy Transfer/Sequencing/Massively Parallel Sequencing