Beta Globin (HBB) Sequencing and Deletion/Duplication

Variants in the beta (β)-globin gene (HBB) can result in anemia, β thalassemia or sickling disorders of varying severity. Typical testing strategy is as follows:

- Initial testing: screen for abnormal hemoglobin (Hb) variants using high-performance liquid chromatography (HPLC) and electrophoresis
- Secondary testing: molecular analysis to identify or confirm abnormal Hb variant(s) detected by HPLC or Hb electrophoresis

Disease Overview

Prevalence

- ~5% of the world’s population carries clinically important Hb variants
- 300,000 individuals with a severe hemoglobinopathy are born annually
- β thalassemias are most commonly observed in individuals from southern Europe, northern Africa, and India

Symptoms

<table>
<thead>
<tr>
<th>Phenotypes caused by HBB variants</th>
<th>Characteristics</th>
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<tbody>
<tr>
<td>Thalassemia: decrease in protein produced</td>
<td>β thalassemia minor (trait)</td>
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<td>- Usually clinically asymptomatic, mild anemia may be present</td>
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<td>- Minor hematologic anomalies, including reduced MCV and elevated HbA2</td>
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<td>β thalassemia major</td>
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<td>- Associated with severe microcytic anemia and hepatosplenomegaly</td>
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<td>- Affected individuals are transfusion dependent</td>
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<td>β thalassemia intermedia</td>
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<td>- Milder clinical presentation than β thalassemia major</td>
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MCV, mean corpuscular volume
Indications for Ordering

- Confirm carrier status or diagnosis of β thalassemia or β globinopathy in individual with clinical findings or family history of β thalassemia or hemoglobinopathy
- Identify or confirm abnormal Hb variant(s) detected by HPLC or Hb electrophoresis

Test Description

Polymerase chain reaction (PCR) amplification and bidirectional sequencing of HBB coding region, intron/exon boundaries, proximal promoter and untranslated regions, and deep intronic variants (IVS-II-654, IVS-II-705, IVS-II-745)

Beta Globin (HBB)
Deletion/Duplication 2010113
Method: Multiplex Ligation-dependent Probe Amplification

Detects large deletions of the β-globin gene cluster associated with β thalassemia or HPFH

Indications for Ordering

- Confirm carrier status or diagnosis of β thalassemia or β globinopathy in individual with clinical findings or family history of β thalassemia or hemoglobinopathy
- Assess for deletional HPFH in individuals with elevated Hb F

Test Description

Multiplex ligation-dependent probe amplification (MLPA) of the β-globin gene cluster (HBB, HBD, HBG1, HBG2, HBE1) and its locus control region

Hemoglobin Evaluation with Reflex to Electrophoresis and/or RBC Solubility 0050610
Method: High Performance Liquid Chromatography/Electrophoresis/RBC Solubility

Effective test for screening and follow up of individuals with known hemoglobinopathies
Test Interpretation

Sensitivity/Specificity

- Clinical sensitivity: 99% (~97% by sequencing and ~2% by deletion analysis) for β thalassemia and hemoglobinopathies associated with the HBB gene
- Analytical sensitivity: 99%

Results

- Pathogenic HBB gene variant(s) detected
  - Heterozygous
    - Carrier of a structurally abnormal Hb or β thalassemia, depending on the specific variant identified
  - Homozygous or compound heterozygous
    - Variably affected, depending on the specific variant(s) identified
- No pathogenic HBB gene variants detected
  - Significantly decreases possibility of β thalassemia or β globinopathy
  - Clinically benign structural variants predicted to produce an abnormal electrophoresis/HPLC result will be reported

Limitations

- Diagnostic errors can occur due to rare sequence variations
- Breakpoints of large deletions and duplications will not be determined
- Precise clinical phenotype associated with a particular deletion may not be known (eg, HPFH vs. delta-beta [δ-β] thalassemia)
- Intragenic deletions in the β-globin cluster genes, other than HBB, may not be detected
- Does not assess for sequence variants within the coding or regulatory regions of the HBD, HBG1, HBG2, and HBE1 genes

Related Tests

Hemoglobin Evaluation Reflexive Cascade 2005792
Method: High Performance Liquid Chromatography/Electrophoresis/RBC Solubility/Polymerase Chain Reaction/Fluorescence Resonance Energy Transfer/Sequencing

Familial Mutation, Targeted Sequencing, Fetal 2001980
Method: Polymerase Chain Reaction/Sequencing

Familial Mutation, Targeted Sequencing 2001961
Method: Polymerase Chain Reaction/Sequencing

Beta Globin (HBB) Sequencing, Fetal 0050388
Method: Polymerase Chain Reaction/Sequencing