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>Phenotype</th>
<th>Characteristics</th>
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<tbody>
<tr>
<td>Thalassemia: decrease in protein</td>
<td>β thalassemia minor (trait)</td>
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<tr>
<td>produced</td>
<td>• Usually clinically asymptomatic, mild anemia may be present</td>
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β thalassemia minor (trait)

- Usually clinically asymptomatic, mild anemia may be present
- Minor hematologic anomalies, including reduced MCV and elevated HbA2

β thalassemia major

- Associated with severe microcytic anemia and hepatosplenomegaly
- Affected individuals are transfusion dependent

β thalassemia intermedia

- Milder clinical presentation than β thalassemia major

Tests to Consider

Beta Globin (HBB) Sequencing and Deletion/Duplication 2010117

Method: Polymerase Chain Reaction/Sequencing/Multiplex Ligation-dependent Probe Amplification

Preferred test for molecular confirmation of β thalassemia or a hemoglobinopathy involving the β-globin gene.

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 regions, intron/exon boundaries, 5’ proximal promoter and untranslated region, 3’ polyadenylation signal, and intronic positions c.93-21 (IVS-I-110), c.316-197 (IVS-II-654), c.316-146 (IVS-II-705), c.316-106 (IVS-II-745), and c.316-86_316-85 (IVS-II-765 L1)

- Includes analysis of the following pathogenic intronic variants: c.93-21G>A, c.316-197C>T, c.316-146T>G, c.316-106C>G, and c.316-86_316-85insCTGCTTTTATTT

Beta Globin (HBB) Gene Sequencing 0050578

Method: Polymerase Chain Reaction/Sequencing

Molecular confirmation of a suspected structural hemoglobinopathy or β thalassemia

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

MCV, mean corpuscular volume
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 regions, intron/exon boundaries, 5' proximal promoter and untranscribed region, 3' polyadenylation signal, and intronic positions c.93-21 (IVS-I-110), c.316-197 (IVS-II-654), c.316-146 (IVS-II-705), c.316-106 (IVS-II-745), and c.316-86_316-85 (IVS-II-765 L1)

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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

Genetics

Gene
HBB

Inheritance
Autosomal recessive (typically)

Structure/Function
- Major adult Hb (HbA): composed of two β-globin chains and two α-globin chains
- Normal adults have two functional β-globin genes (HBB) and four functional α-globin genes (two copies each of HBA1 and HBA2)
- β-globin chains with different variants may interact to alleviate or exacerbate the effects of the individual variants
  - Variants in HBB gene can result in formation of a structurally abnormal protein or decrease the amount of protein produced
  - Certain HBB deletions impair the developmental switch from fetal to adult Hb, resulting in hereditary persistence of fetal Hb

Variants
>500 β-globin variants
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