Alpha Thalassemia

Indications for Ordering

- Carrier screening
  - Healthy individuals of African, Mediterranean, Middle Eastern, and Southeast Asian descent
  - Individuals with a family history of α thalassemia
  - Individuals with reproductive partners who are affected with, or carriers of, α thalassemia
  - Individuals with microcytosis and no identified iron deficiency
- Diagnostic testing
  - Confirm a clinical diagnosis of hemoglobin (Hb) Bart hydrops fetalis syndrome or Hb H disease
  - Confirm the identity of a variant detected by Hb evaluation that may be pathogenic or benign

Test Description

- Targeted testing for common deletions
  - PCR/gel electrophoresis
  - 7 common deletions of HBA1 and HBA2 genes (-α3.7, -α4.2, -(α)20.5, --SEA, --MED, --FIL, and --THAI)
- Deletion/duplication analysis by multiplex ligation-dependent probe amplification of the α-globin gene cluster (HBZ, HBM, HBA1, HBA2, HBQ1) and its HS-40 regulatory region
- Sequencing by PCR amplification followed by bidirectional sequencing of
  - HBA1 and HBA2 coding regions
  - Intron/exon boundaries
  - Proximal promoter regions
  - 5’ and 3’ untranslated regions
  - Polyadenylation signals

Tests to Consider

Typical testing strategy

- Molecular analysis for large deletions in the α-globin genes
- Consider α-globin gene sequencing if
  - Deletion testing does not explain the clinical phenotype
  - OR
  - Hb evaluation suggests the presence of an abnormal α-chain variant

Primary tests

- Alpha Globin (HBA1 and HBA2) Deletion/Duplication 2011622
  - 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 H
- Alpha Thalassemia (HBA1 and HBA2) 7 Deletions 0051495
  - Acceptable first-tier genetic test for confirmation of suspected α thalassemia or α thalassemia trait
- Alpha Globin (HBA1 and HBA2) Sequencing and Deletion/Duplication 2011708
  - Comprehensive genetic test for detection of α thalassemia or α thalassemia trait
  - Detects deletional and nondeletional variants in HBA1 and HBA2

Related tests

- Hemoglobin Evaluation Reflexive Cascade 2005792
  - Optimal test for the initial and confirmatory diagnosis of any suspected hemoglobinopathy
  - Cascade reflex testing may include electrophoresis, solubility testing, and/or molecular analysis of the globin genes
- Hemoglobin Evaluation with Reflex to Electrophoresis and/or RBC Solubility 0050610
  - Effective test for screening and follow up of individuals with known hemoglobinopathies
  - Optimal test for the initial diagnosis of a suspected hemoglobinopathy is Hemoglobin Evaluation Reflexive Cascade
- Familial Mutation, Targeted Sequencing 2001961
  - Useful when a pathogenic familial variant identifiable by sequencing is known
- Familial Mutation, Targeted Sequencing, Fetal 2001980
  - Fetal test to detect a previously characterized variant in a family member

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 Hb H disease
  - More frequent in Southeast Asian, Asian Indian, and Mediterranean populations than in African populations
Symptoms
- α thalassemia silent carrier
  - Typically asymptomatic
    - Borderline anemia or mild microcytosis may be present
    - Often misdiagnosed as iron deficiency
  - Normal Hb electrophoresis
- α thalassemia trait
  - Mild microcytic anemia may be present
  - Often misdiagnosed as iron deficiency
  - Normal Hb electrophoresis
- Hb H disease
  - Moderate to severe form of α thalassemia
  - Moderate microcytic hypochromic anemia
  - Hemolysis with Heinz bodies
  - Splenomegaly
  - Rare extramedullary hematopoiesis
  - Propensity for acute hemolysis after oxidative stress, drug therapy, or infection
- Hb Bart hydrops fetalis syndrome
  - Most severe form of α thalassemia
  - Fetus
    - Lethal in fetal or early neonatal period
    - Generalized edema, ascites, pleural and pericardial effusions
    - Severe hypochromic anemia
    - Usually detected on ultrasound at 22-28 weeks gestation
  - Maternal complications during pregnancy
    - Preeclampsia
    - Polyhydramnios or oligohydramnios
    - Antepartum hemorrhage
    - Premature delivery

Physiology
- Typically, individuals have 4 functioning α-globin genes (αα/αα)
  - 2 genes, HBA1 and HBA2, are present on each copy of chromosome 16
  - α-globin chains function as subunits of fetal Hb (Hb F – α2γ2) and adult Hb (Hb A – α2β2)
- Genotype/phenotype correlations in α-thalassemia are complex and may be influenced by coinheritance of other Hb variants or α-globin gene duplications
- α thalassemia silent carrier
  - 1 nonfunctional α-globin gene (-α/αα)
- α thalassemia trait
  - 2 nonfunctional α-globin genes in trans (-α/-α) or cis (-/-αα)
- Hb H disease
  - 3 nonfunctional α-globin genes (-/-α)
  - Hb Bart hydrops fetalis syndrome
  - 4 nonfunctional α-globin genes (-/-)

Genetics

Genes – HBA1 and HBA2

Inheritance – autosomal recessive

Variants
- HBA1 and HBA2 large gene deletions account for up to 90% of α thalassemia
  - -α3.7 and -α4.2 deletions result in the deletion of a single gene
  - -α20.5, --SEA, --MED, --FIL, and --THAI deletions result in the deletion of the HBA1 and HBA2 genes from the same chromosome
- Point variants and regulatory region variants occur mainly in HBA2 and account for up to 10% of causative variants
  - Nondeletional variants include
    - Point variants that inactivate the gene
    - Small insertions/deletions
    - Variants that result in unstable α-globin protein (eg, Hb Constant Spring)
  - 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
- α-globin gene triplications result in three active α-globin genes on a single chromosome
  - Typically benign
  - May alter expected clinical phenotypes and hematological features when co-inherited with β thalassemia

Test Interpretation

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 – ≤90%, depending on ethnicity (Origa, 2013)
  - Sequencing – ≤9%, depending on ethnicity (Origa, 2013)

Results and limitations – see table

References
### Alpha Thalassemia (HBA1 and HBA2) 7 Deletions

**Negative result**
- No common α-globin gene deletions were detected
  - Risk for α thalassemia is reduced but not excluded

**Positive result**
- Predicted genotype (-α/αα)
  - Individual is predicted to be a silent carrier
- Predicted genotype (-α/-α) or (-/-αα)
  - Individual is predicted to have α thalassemia trait
- Predicted genotype (--/-α)
  - Individual is predicted to be affected with Hb H disease
- Predicted genotype (--/-)
  - Result is consistent with Hb Bart hydrops fetalis syndrome

**Inconclusive result**
- Deletion or duplication of unknown clinical significance detected

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

**Negative result**
- No large α-globin deletions or duplications were detected
  - Risk for α thalassemia is reduced but not excluded

**Positive result**
- Predicted genotype (-α/αα)
  - Individual is predicted to be a silent carrier
- Predicted genotype (-α/-α) or (-/-αα)
  - Individual is predicted to have α thalassemia trait
- Predicted genotype (--/-α)
  - Individual is predicted to be affected with Hb H disease
- Predicted genotype (--/-)
  - Result is consistent with Hb Bart hydrops fetalis syndrome
- Predicted genotype (ααα/αα)
  - An extra functional α-globin gene present

**Inconclusive result**
- Variant of unknown clinical significance detected

### Alpha Globin (HBA1 and HBA2) Sequencing

**Negative result**
- No pathogenic variants were detected
  - Risk for α thalassemia is reduced
  - Large deletions of the α-globin genes, which account for the majority of variants, are not detected by sequencing

**Positive result**
- One pathogenic variant detected
  - Individual is predicted to be a carrier of α thalassemia
  - A more severe disorder is possible if another undetected α-globin variant is present
- Two pathogenic variants detected
  - Individual is predicted to be a carrier of α thalassemia; mild microcytic anemia often present
  - homozygosity or compound heterozygosity for nondeleitional variants results rarely in Hb H disease

**Inconclusive result**
- Large deletions/duplications and some variants of the regulatory regions will not be detected
- The phase of identified variants may not be determined
- Diagnostic errors can occur due to rare sequence variations
- Sequencing of both HBA1 and HBA2 may not be possible in individuals harboring large α-globin deletions on both alleles
- Rare syndromes associated with α thalassemia, such as ATR-X and ATR-16, will not be detected

### Limitations

- Rare α-globin gene deletions, nondeleitional variants, gene duplications and variants of the regulatory region will not be detected
- α-globin gene duplications will not be detected
- Diagnostic errors can occur due to rare sequence variations
- Rare syndromic or acquired forms of α thalassemia will not be detected
- Breakpoints of large deletions/duplications will not be determined; therefore, it may not be possible to distinguish variants of similar size
- This assay does not assess for nondeleitional variants within the coding or regulatory regions of the α-globin cluster genes
- 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
- Diagnostic errors can occur due to rare sequence variations

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