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
  - -(α)20.5, --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

- Origa R, Moi P, et al. Alpha-Thalassemia. 2005 Nov 1 [Updated 2013 Nov 21]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1435/
- Tan AS, Quah TC, et al. A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for α-thalassemia. Blood. 2001;98(1):250-251

	Alpha Thalassemia (*HBA1* and *HBA2*) 7 Deletions	**Alpha Globin (*HBA1* and *HBA2*) Deletion/Duplication**	**Alpha Globin (*HBA1* and *HBA2*) Sequencing**
Negative result	• No common α-globin gene deletions were detected o Risk for α thalassemia is reduced but not excluded	• No large α-globin deletions or duplications were detected o Risk for α thalassemia is reduced but not excluded	• No pathogenic variants were detected o Risk for α thalassemia is reduced o Large deletions of the α-globin genes, which account for the majority of variants, are not detected by sequencing
Positive result	• Predicted genotype (-α/αα) o Individual is predicted to be a silent carrier • Predicted genotype (-α/-α) or (--/αα) o Individual is predicted to have α thalassemia trait • Predicted genotype (--/-α) o Individual is predicted to be affected with Hb H disease • Predicted genotype (--/--) o Result is consistent with Hb Bart hydrops fetalis syndrome	• Predicted genotype (-α/αα) o Individual is predicted to be a silent carrier • Predicted genotype (-α/-α) or (--/αα) o Individual is predicted to have α thalassemia trait • Predicted genotype (--/-α) o Individual is predicted to be affected with Hb H disease • Predicted genotype (--/--) o Result is consistent with Hb Bart hydrops fetalis syndrome • Predicted genotype (ααα/αα) o An extra functional α-globin gene present	• One pathogenic variant detected o Individual is predicted to be a carrier of α thalassemia o A more severe disorder is possible if another undetected α-globin variant is present • Two pathogenic variants detected o Individual is predicted to be a carrier of α thalassemia; mild microcytic anemia often present o Homozygosity or compound heterozygosity for nondeletional variants results rarely in Hb H disease
Inconclusive result		Deletion or duplication of unknown clinical significance detected	Variant of unknown clinical significance detected
Limitations	• Rare α-globin gene deletions, nondeletional 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 nondeletional 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	• 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