Alpha Thalassemia

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

Phenotype	Associated Symptoms
α Thalassemia silent carrier	Typically asymptomatic, though 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
HbH 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

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 (Extended TAT as of 11/20/20-no referral available) 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 (Temporary Referral as of 05/10/21) 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 to familial *HBA1*/*HBA2* deletions or HbCS variant

Phenotype	Associated Symptoms
Hb Bart hydrops fetalis syndrome	Most severe form of α thalassemia Risk for 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

- 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 in absence of a concurrent *HBA2* deletion.

See Related Tests

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.

Phenotype	Genotype(s)
α Thalassemia silent carrier	-α/αα
α Thalassemia trait	-α/-α --/αα
HbH disease	--/-α
Hb Bart hydrops fetalis syndrome	--/--

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 HBcS)
    - 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[1]
    - Sequencing: up to 15%, varies by ethnicity[1]

Results

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

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

1. Origa R, Moi P. Alpha-thalassemia. In: Adam MP, Ardinger HH, Pagon RA, et al, editors. GeneReviews, University of Washington; 1993-2021. [Last update: Oct 2020; Accessed: Jan 2021]

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

Familial Mutation, Targeted Sequencing 2001961

Method: Polymerase Chain Reaction/Sequencing

Familial Mutation, Targeted Sequencing, Fetal 2001980

Method: Polymerase Chain Reaction/Sequencing

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