

Alpha Thalassemia: *HBA1* and *HBA2* Common Gene Deletions or Sequencing

TO DETERMINE CARRIER OR AFFECTED STATUS FOR ALPHA-THALASSEMIA

Disease Overview

- Alpha-thalassemia, the most common inherited disorder of hemoglobin worldwide, is caused by decreased or absent production of the alpha-globin chain of hemoglobin (Hb).
- Clinical symptoms are related to inadequate Hb production and accumulation of beta- and/or gamma-globin subunits.
- Normal individuals have four functioning alpha-globin genes ($\alpha\alpha/\alpha\alpha$). Two genes—alpha-1 (*HBA1*) and alpha-2 (*HBA2*)—are present on each copy of chromosome 16.
 - Alpha-globin chains function as subunits of fetal hemoglobin (Hb F: $\alpha_2\gamma_2$) and adult hemoglobin (Hb A: $\alpha_2\beta_2$).
- Classic deletional alpha-thalassemia results from large deletions involving the *HBA1* and/or the *HBA2* gene. Subtypes of deletional alpha-thalassemia include:
 - Hb Bart (γ_4) hydrops fetalis syndrome
 - Deletion of all four alpha-globin genes ($---/---$)
 - Clinical findings in the fetus include generalized edema, ascites, pleural and pericardial effusions, and severe hypochromic anemia, as well as fetal or perinatal death.
 - Maternal complications during pregnancy may include pre-eclampsia, polyhydramnios or oligohydramnios, antepartum hemorrhage, and premature delivery.
 - Hemoglobin H (Hb H) disease
 - Deletion of three alpha-globin genes ($---/\alpha$).
 - Associated with the presence of Hb H (β_4); alternatively, Hb Bart in a neonate.
 - Clinical findings include moderate microcytic hypochromic anemia, hemolysis with Heinz bodies, splenomegaly, rare extramedullary hematopoiesis, and propensity for acute hemolysis after oxidative stress, drug therapy, or infection.
 - Alpha-thalassemia trait
 - Deletion of two alpha-globin genes ($-\alpha/-\alpha$ or $---/\alpha\alpha$)
 - Mild microcytic anemia may be present; normal hemoglobin electrophoresis; often misdiagnosed as iron deficiency.
 - For prenatal counseling, it is important to define whether the non-functioning alpha-globin genes lie on the same chromosome (cis configuration) or the opposite chromosome (trans configuration). When both parents carry a cis deletion of *HBA1* and *HBA2* ($---/\alpha\alpha$), the risk for hydrops fetalis associated with Hb Bart in their offspring may be 1:4 (homozygosity for certain cis deletions results in embryonic lethality).
 - Alpha-thalassemia silent carrier
 - Deletion of a single alpha-globin gene ($-\alpha/\alpha\alpha$)
 - Typically asymptomatic; borderline anemia or mild microcytosis may be present.
 - Normal hemoglobin electrophoresis; often misdiagnosed as iron deficiency

- Non-deletional alpha-globin mutations may be pathogenic or benign; either type of mutation may result in an abnormal protein detectable by hemoglobin evaluation. Pathogenic non-deletional mutations often have a more severe effect than single gene deletions.
- Genotype/phenotype correlations in alpha-thalassemia are complex and may be influenced by co-inheritance of other hemoglobin variants or alpha-globin gene duplications.

Epidemiology

- High carrier frequencies in Mediterranean (1/30–50), Southeast Asian (1/20), Middle Eastern, African, and African-American (1/3) populations.
- Hb Bart hydrops fetalis syndrome occurs more often in Southeast Asian, Asian Indian, and Mediterranean populations than in African populations due to the rarity of cis deletions ($---/\alpha\alpha$) in Africa.

Genetics

- Autosomal recessive inheritance
- Ninety-five percent of alpha-thalassemia is caused by *HBA1* and *HBA2* gene deletions; point mutations and regulatory region mutations are relatively rare.
- The $-\alpha_3.7$ and $-\alpha_4.2$ alpha-globin gene deletions result in the deletion of a single gene; the $-(\alpha)20.5$, $---SEA$, $---MED$, $---FIL$, and $---THAI$ deletions result in the deletion of the *HBA1* and *HBA2* genes from the same chromosome.
- Pathogenic non-deletional mutations occur mainly in *HBA2*. Non-deletional mutations include point mutations that inactivate the gene, small insertions/deletions, and mutations that result in unstable alpha-globin protein.
- Rare disorders associated with alpha-thalassemia include alpha-thalassemia X-linked mental retardation syndrome (ATR-X), which is associated with *ATRX* gene mutations, and alpha-thalassemia/mental retardation syndrome, chromosome 16-related (also known as ATR-16 syndrome), which is caused by large deletions that include multiple genes of chromosome 16p.

Indications for Ordering

- Alpha Thalassemia (*HBA1* & *HBA2*) 7 Deletions
 - Carrier screening for healthy individuals of African, Mediterranean, Middle Eastern and Southeast Asian descent
 - Carrier screening for individuals with a family history of alpha-thalassemia or for individuals with reproductive partners who are affected with, or carriers of, alpha-thalassemia

- To establish diagnosis and confirm carrier status for individuals with microcytosis and no identified iron deficiency
- To confirm a clinical diagnosis of Hb Bart hydrops fetalis syndrome or Hb H disease
- Alpha Globin (*HBA1* & *HBA2*) Sequencing
 - To confirm a clinical diagnosis of alpha-thalassemia when *HBA1* and *HBA2* deletion testing has detected the inactivation of two or fewer alpha-globin genes
 - To confirm the identity of a variant detected through hemoglobin evaluation that may be pathogenic or benign

Additional Ordering Notes

- For targeted sequencing of a previously identified, familial alpha-globin sequence variant, order Familial Mutation, Targeted Sequencing (2001961).
- For optimal test interpretation, please submit a “Patient History for Hemoglobinopathy/Thalassemia Testing” form detailing the patient’s clinical findings, family history, and ethnicity.

Interpretation

- Alpha Thalassemia (*HBA1* & *HBA2*) 7 Deletions
 - Negative: No common alpha-globin gene deletions were detected; the risk for alpha-thalassemia is reduced but not excluded.
 - Positive:
 - Predicted genotype $(-a/aa)$ or $(-a/-a)$ or $(-/-a\ a)$: This individual is predicted to have alpha-thalassemia trait or to be a silent carrier.
 - Predicted genotype $-/-a$: This individual is predicted to be affected with Hb H disease.
 - Predicted genotype $-/-$: This result is consistent with Hb Bart hydrops fetalis syndrome.
- Alpha Globin (*HBA1* & *HBA2*) Sequencing
 - Negative: No pathogenic mutations were detected; the risk for alpha-thalassemia is reduced. Large deletions of the alpha-globin genes, which account for the majority of mutations, are not detected by sequencing.
 - Positive:
 - One pathogenic mutation detected: Individuals with a single pathogenic, non-deletional alpha-globin gene mutation are predicted to be carriers of alpha-thalassemia. A more severe disorder is possible if another undetected alpha-globin mutation is present.
 - Two pathogenic mutations detected: Individuals with two pathogenic, non-deletional alpha-globin gene mutations (on opposite chromosomes) often have mild microcytic anemia; homozygosity or compound heterozygosity for non-deletional mutations may rarely result in Hb H disease.
 - Mutation of unknown clinical significance: A mutation of unknown clinical significance was identified.

Methodology and Limitations

- Alpha Thalassemia (*HBA1* & *HBA2*) 7 Deletions
 - Common deletions of the *HBA1* and the *HBA2* genes ($-a3.7$, $-a4.2$, $-(a)20.5$, $-SEA$, $-MED$, $-FIL$, and $-THAI$) are assayed by polymerase chain reaction and gel electrophoresis.
 - Clinical sensitivity is up to 95%, depending on ethnicity.
 - Analytical sensitivity and specificity are 99%.

- Rare alpha-globin gene deletions, non-deletional mutations, and mutations of the regulatory region will not be detected.
- Alpha-globin gene duplications will not be detected.
- Rare diagnostic errors may occur due to primer-site mutations.
- Alpha Globin (*HBA1* & *HBA2*) Sequencing
 - PCR amplification followed by bidirectional sequencing of the *HBA1* and *HBA2* coding regions, intron/exon boundaries, proximal promoter regions, 5’ and 3’ untranslated regions, and polyadenylation signals.
 - Clinical sensitivity is up to 10%, depending on ethnicity.
 - Analytical sensitivity and specificity are 99%.
 - Large deletions and some mutations of the regulatory regions will not be detected.
 - Gene duplications will not be detected.
 - The phase of most identified mutations will not be determined.
 - Rare diagnostic errors can occur due to primer-site mutations.
 - This test is not able to identify sequence variants in an alpha-globin gene in cis with the common 3.7 Kb deletion ($-a3.7$). Therefore, sequencing is not possible in individuals homozygous for the 3.7 Kb deletion; individuals heterozygous for the 3.7 Kb deletion will appear homozygous for sequence variants present on the non-deleted allele.

Related Tests

- Hemoglobin Evaluation with Reflex to Electrophoresis and/or RBC Solubility (0050610)
- Hemoglobin Bart (0050528)
- Beta Globin (*HBB*) Gene Sequencing (0050578)
- Beta Globin (*HBB*) HbS, HbC, & HbE Mutations (0051421)
- Familial Mutation, Targeted Sequencing (2001961)
- Familial Mutation, Targeted Sequencing, Fetal (2001980)

References

1. Tan ASC, et al. A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for α -thalassemia. *Blood*. 2001;98(1):250–251.
2. Weatherall DJ. Disorders of globin synthesis: the thalassemias. In: Lichtman MA, et al., eds. *Williams Hematology*. 7th ed. New York, NY: McGraw-Hill; 2006:633–666.
3. Galanello R, Cao A. Alpha-Thalassemia. 2005 (Updated 2011). In: GeneReviews at GeneTests Medical Genetics Information Resource (database online). Copyright, University of Washington, Seattle. 1997–2012. Available at genetests.org. Accessed May 7, 2012.
4. Higgs DR, Weatherall DJ. The alpha thalassemias. *Cell Mol Life Sci*. 2009;66:1154–1162.

Test Information

0051495 **Alpha Thalassemia (*HBA1* & *HBA2*) 7 Deletions**
2001582 **Alpha Globin (*HBA1* & *HBA2*) Sequencing**

For specific collection, transport, and testing information, refer to the ARUP website at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

AUTHORS

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