

Genetic Testing for α -Thalassemia

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Next Review: January 2020

Last Review: March 2019

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Alpha-thalassemia represents a group of clinical syndromes of varying severity characterized by hemolytic anemia and ineffective hematopoiesis. Genetic defects in any or all of four α -globin genes are causative of these syndromes.

MEDICAL POLICY CRITERIA

- I. Prenatal (fetal) genetic testing for α -thalassemia may be considered **medically necessary**.
- II. Preconception (carrier) testing for α -thalassemia in prospective parents may be considered **medically necessary** when both reproductive partners have evidence of possible α -thalassemia (including α -thalassemia minor, hemoglobin H disease [α -thalassemia intermedia], or α -thalassemia major) based on biochemical testing (see Policy Guidelines section).
- III. Genetic testing to confirm a diagnosis of α -thalassemia is considered **not medically necessary**.
- IV. Genetic testing of patients with hemoglobin H disease (alpha-thalassemia intermedia) to determine prognosis is considered **investigational**.

IV. Genetic testing for α -thalassemia in other clinical situations (excluding prenatal testing) is considered **investigational**.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

SUBMISSION OF DOCUMENTATION:

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or variant(s) being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test:
 - o History and physical exam including any relevant diagnoses related to the genetic testing
 - o Conventional testing and outcomes
 - o Conservative treatments, if any

Strategies for testing may include testing for individual genes or in combination, such as in a panel.

Alpha-thalassemias include:

- Thalassemia trait (α -thalassemia minor)
- Hemoglobin H Disease (α -thalassemia intermedia)
- Hemoglobin Bart's (α -thalassemia major, hydrops fetalis)

The probability of a pregnancy with hemoglobin Bart's (α -thalassemia major) is dependent on the specific genotype found in each parent.

This policy does not address prenatal (in utero or preimplantation) genetic testing for α -thalassemia.

BIOCHEMICAL TESTING

Biochemical testing to determine whether α -thalassemia is present should be the first step in evaluating the presence of the condition. Biochemical testing consists of complete blood count (CBC), microscopic examination of the peripheral blood smear, and hemoglobin electrophoresis. In silent carriers and in α -thalassemia trait, the hemoglobin electrophoresis will most likely be normal. However, there should be evidence of possible α -thalassemia minor on the CBC and peripheral smear.

CROSS REFERENCES

1. [Genetic and Molecular Diagnostic Testing](#), Genetic Testing, Policy No. 20
2. [Evaluating the Utility of Genetic Panels](#), Genetic Testing, Policy No. 64
3. [Reproductive Carrier Screening for Genetic Diseases](#), Genetic Testing, Policy No. 81

BACKGROUND

ALPHA-THALASSEMIA

Alpha-thalassemia is a common genetic disorder, affecting approximately 5% of the world's population.^[1] The frequency of variants is highly dependent on ethnicity, with the highest rates seen in Asians, and much lower rates in Northern Europeans. The carrier rate is estimated to be 1 in 20 in Southeast Asians, 1 in 30 for Africans, and between 1 in 30 and 1 in 50 for individuals of Mediterranean ancestry. By contrast, for individuals of northern European ancestry, the carrier rate is less than 1 in 1000.

Physiology

Hemoglobin, which is the major oxygen-carrying protein molecule of red blood cells (RBCs), consists of two α -globin chains and two β -globin chains. Alpha-thalassemia refers to a group of syndromes that arise from deficient production of α -globin chains. Deficient α -globin production leads to an excess of β -globin chains, which results in anemia by a number of mechanisms^[2]:

- Ineffective erythropoiesis in the bone marrow.
- Production of nonfunctional hemoglobin molecules.
- Shortened survival of RBCs due to intravascular hemolysis and increased uptake of the abnormal RBCs by the liver and spleen.

The physiologic basis of α -thalassemia is a genetic defect in the genes coding for α -globin production. Each individual carries four genes that code for α -globin (two copies each of *HBA1* and *HBA2*, located on chromosome 16), with the wild genotype (normal) being aa/aa . Genetic variants may occur in any or all of these four α -globin genes. The number of genetic variants determines the phenotype and severity of the α -thalassemia syndromes. There are four different syndromes, which are classified below.

Silent Carrier

Silent carrier (α -thalassemia minima) arises from one of four abnormal α genes ($\alpha\alpha/\alpha-$), and is a silent carrier state. A small amount of abnormal hemoglobin can be detected in the peripheral blood, and there may be mild hypochromia and microcytosis present, but there is no anemia or other clinical manifestations.

Thalassemia Trait

Thalassemia trait (α -thalassemia minor), also called α -thalassemia trait, arises from the loss of two α -globin genes, resulting in one of two genotypes ($\alpha\alpha/--$, or $\alpha-/α-$). Mild anemia is present, and RBCs are hypochromic and microcytic. Clinical symptoms are usually absent and, in most cases, the hemoglobin electrophoresis is normal.

Hemoglobin H Disease

Hemoglobin H (HbH) disease (α -thalassemia intermedia) results from three abnormal α -globin genes (α -/--), resulting in moderate-to-severe anemia. In HbH disease, there is an imbalance in α - and β -globin gene chain synthesis, resulting in the precipitation of excess β chains into the characteristic hemoglobin H, or β -tetramer. This condition has marked phenotypic variability, but most individuals have mild disease and live a normal life without medical intervention.^[3]

A minority of individuals may develop clinical symptoms of chronic hemolytic anemia. They include neonatal jaundice, hepatosplenomegaly, hyperbilirubinemia, leg ulcers, and premature development of biliary tract disease. Splenomegaly can lead to the need for splenectomy, and transfusion support may be required by the third to fourth decade of life. It has been estimated that approximately 25% of patients with HbH disease will require transfusion support during their lifetime.^[1] In addition, increased iron deposition can lead to premature damage to the liver and heart. Inappropriate iron therapy and oxidant drugs should be avoided in patients with HbH disease.

There is an association between genotype and phenotype among patients with HbH disease. Individuals with a nondeletion variant typically have an earlier presentation, more severe anemia, jaundice, and bone changes, and more frequently require transfusions.^[4]

Hemoglobin Bart's

Hemoglobin Bart's (α -thalassemia major) results from variants in all four α -globin genes (----), which prevents production of α -globin chains. This condition causes hydrops fetalis, which often leads to intrauterine death or death shortly after birth. There are also increased complications during pregnancy for a woman carrying a fetus with hydrops fetalis. They include hypertension, preeclampsia, antepartum hemorrhage, renal failure, premature labor, and abruption placenta.^[1]

Genetic Testing

A number of different types of genetic abnormalities are associated with α -thalassemia. More than 100 genetic variants have been described. Deletion of one or more of the α -globin chains is the most common genetic defect. This type of genetic defect is found in approximately 90% of cases.^[4] Large genetic rearrangements can also occur from defects in crossover and/or recombination of genetic material during reproduction. Point mutations in one or more of the α genes that impair transcription and/or translation of the α -globin chains.

Testing is commercially available through several genetic labs. Targeted variant analysis for known α -globin gene variants can be performed by polymerase chain reaction (PCR).^[4] PCR can also be used to identify large deletions or duplications. Newer testing methods have been developed to facilitate identification of α -thalassemia variants, such as multiplex amplification methods and real-time PCR analysis.^[5-7] In patients with suspected α -thalassemia and a negative PCR test for genetic deletions, direct sequence analysis of the α -globin locus is generally performed to detect point variants.^[4]

REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA). Genetic testing for α -thalassemia is available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA

for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature^[8] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

GENETIC TESTING FOR ALPHA-THALASSEMIA

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a variant that is present or in excluding a variant that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

The published literature on genetic testing for α -thalassemia consists primarily of reports describing the molecular genetics of testing, the types of variants encountered, and genotype-phenotype correlations.^[5,6,9-13]

Analytic Validity

A variety of testing methods can be used to evaluate the two genes related to α -globin production, *HBA1* and *HBA2*, including sequence analysis of the entire coding region, targeted variant analysis via polymerase chain reaction (PCR), and deletion/duplication analysis. Therefore, the analytic validity depends on the method used, but would generally be expected to be high.

One 2016 study identified evaluated the reproducibility and accuracy of a PCR-based multicolor melting curve analysis method for detecting common nondeletional variants in the *HBA2* gene from 700 whole blood samples.^[14] Reproducibility of the assay was high. In the clinical samples, there was 100% concordance between the 20 genotypes identified and the genotyping method. Petropoulou (2015) evaluated a PCR-based high-resolution melting curve analysis of duplicated areas of the *HBA1* and *HBA2* genes with novel nondeletion variants.^[15] The study included 62 samples with previously identified novel variants and 18 normal controls; the melting curve analysis was able to distinguish at least 80% of novel homozygote samples detected by earlier generation tests.

Clinical Validity

Clinical validity is expected to be high when the causative variant is a large deletion of one or more α -globin gene, as PCR testing is generally considered highly accurate for this purpose. When a point variant is present, the clinical validity is less certain.

Henderson (2016) reported on a retrospective study of genotype and phenotype correlations of the novel thalassemia and abnormal hemoglobin variants identified after adoption of routine DNA sequencing of α - and β -globin genes for all U.K. samples referred for evaluation of hemoglobinopathy for the preceding 10 years.^[16] Of a total of approximately 12,000 samples, 15 novel α -thalassemia variants, 19 novel β -thalassemia variants, and 11 novel β -globin variants were detected.

Clinical Utility

There are several potential areas for clinical utility. Genetic testing can be used to determine the genetic abnormalities underlying a clinical diagnosis of α -thalassemia. It can also be used to define the genetics of α -globin genes in relatives of patients with a clinical diagnosis of α -thalassemia. Preconception (carrier) testing can be performed to determine the likelihood of an offspring with an α -thalassemia syndrome. Prenatal (in utero) testing can also be performed to determine the presence and type of α -thalassemia of a fetus. Prenatal testing is not addressed in this evidence review.

Confirming a Diagnosis

The diagnosis of α -thalassemia can be made without genetic testing. This is first done by analyzing the complete blood count (CBC) and peripheral blood smear, in conjunction with testing for other forms of anemia. Patients with a CBC demonstrating microcytic, hypochromic red blood cell (RBC) indices who are not found to have iron deficiency, have a high likelihood of thalassemia. On peripheral blood smear, the presence of inclusion bodies and target cells is consistent with the diagnosis of α -thalassemia.

Hemoglobin electrophoresis can distinguish between the asymptomatic carrier states and α -thalassemia intermedia (HbH disease) by identifying the types and amounts of abnormal hemoglobin present. In the carrier states, greater than 95% of the hemoglobin molecules are normal (hemoglobin A), with a small minority of hemoglobin A₂ present (1%-3%).^[3] Alpha-thalassemia intermedia is diagnosed by finding a substantial portion of hemoglobin H (1%-30%) on electrophoresis.^[3] In α -thalassemia major, the majority of the hemoglobin is abnormal, in the form of hemoglobin Bart's (85%-90%).^[3]

However, biochemical testing, including CBC and hemoglobin electrophoresis, cannot always reliably distinguish between the asymptomatic carrier state and α -thalassemia trait, because the hemoglobin electrophoresis is typically normal in both conditions. Genetic testing can differentiate between the asymptomatic carrier state (α -thalassemia minima) and α -thalassemia trait (α -thalassemia minor) by measuring the number of abnormal genes present. This distinction is not important clinically because both the carrier state and α -thalassemia trait are asymptomatic conditions that do not require specific medical care treatment. Alpha-thalassemia trait may overlap in RBC indices values with iron deficiency states, so it is important that iron supplementation not be continued unnecessarily in patients with α -thalassemia trait. However, it would be reasonable to make a diagnosis of α -thalassemia trait in a patient with microcytic, hypochromic RBC indices without evidence of iron deficiency, either before or after a trial of iron supplementation. Because the diagnosis of clinically relevant α -thalassemia conditions can usually be made without genetic testing, there is little

utility to genetic testing of a patient with a clinical diagnosis of thalassemia to determine the underlying genetic abnormalities.

Prognostic Testing in Patients with HbH Disease

Among patients with HbH disease, there is heterogeneity in the nature of the variant (i.e., deletional vs. nondeletional), with differences across geographic areas and ethnic groups.^[17] Patients with deletional variants may have a less severe course of illness than those with nondeletional variants.^[17] In a cohort of 147 Thai pediatric patients with HbH disease, those with nondeletional variants were more likely to have pallor after fever, hepatomegaly, splenomegaly, jaundice, short stature, need for transfusions, and gallstones.^[18]

The evidence suggests that different genetic variants leading to α -thalassemia are associated with different prognoses. New treatments for some of the complications of HbH disease that result from ineffective erythropoiesis and iron overload and may differ for genotypes are under development.^[19] However, no evidence was identified to indicate that patient management or outcomes would be changed by prognostic testing.

Preconception (Carrier) Testing

The major benefit of carrier testing is to define the likelihood of α -thalassemia major. Avoiding a pregnancy with α -thalassemia major is of benefit in that a prospective mother will avoid carrying a nonviable pregnancy and will avoid the increased obstetrical complications associated with a fetus with α -thalassemia major.

Carrier screening with biochemical testing is recommended for all patients who are from ethnic groups with a high incidence of α -thalassemia. Biochemical screening consists of a CBC with peripheral smear analysis. If abnormalities are noted, such as anemia, microcytosis, or hypochromia, hemoglobin electrophoresis is then performed to identify the specific types of hemoglobin present. As noted, the hemoglobin electrophoresis may be normal in the asymptomatic carrier and α -thalassemia trait states, but the states may be suspected based on CBC and peripheral smear analysis.

Unlike clinical diagnosis, for carrier testing, it is important to distinguish between α -thalassemia carrier (one abnormal gene) and α -thalassemia trait (two abnormal genes), and important to distinguish between the two variants of α -thalassemia trait, i.e., the $\alpha\alpha/--$ (cis variant) and the $\alpha/-\alpha$ (trans variant). This is important because only when both parents have the $\alpha\alpha/--$ cis variant is there a risk for a fetus with α -thalassemia major.^[20] When both parents are α -thalassemia carriers ($\alpha\alpha/--$), there is a one in four chance that an offspring will have α -thalassemia major and hydrops fetalis. These parents may decide to pursue preimplantation genetic diagnosis in conjunction with in vitro fertilization to avoid a pregnancy with hydrops fetalis.

In this situation, genetic testing has incremental utility over biochemical testing. Although biochemical testing can determine whether a silent carrier/trait syndrome is present, and can distinguish those syndromes from HbH disease, it cannot provide a precise determination of the number or pattern of abnormal alpha genes. As a result, the probability of developing a hemoglobin Bart's fetus cannot be accurately assessed using biochemical screening alone. By contrast, genetic testing can delineate the number of abnormal genes with certainty. In addition, genetic testing can determine whether an α -thalassemia trait exists as the cis ($\alpha\alpha/--$)

variant or the trans (α -/ α -) variant. Using this information from genetic testing, the probability of hemoglobin Bart's can be determined according to Table 1.

Table 1. Probability of Hemoglobin Bart's

Clinical Diagnosis in Parents	Genotype (Parent 1)	Genotype (Parent 2)	Probability of Hemoglobin Bart's
Both parents silent carriers	$\alpha\alpha/\alpha-$	$\alpha\alpha/\alpha-$	0%
One parent silent carrier, 1 parent trait	$\alpha\alpha/\alpha-$	$\alpha-/ \alpha-$	0%
Both parents trait	$\alpha\alpha/--$	$\alpha\alpha/\alpha-$	0%
		$\alpha\alpha/--$	25%
		$\alpha-/ \alpha-$	0%
One parent HbH, 1 parent silent carrier	$\alpha-/ \alpha-$	$\alpha\alpha/\alpha-$	0%
		$\alpha\alpha/--$	25%
		$\alpha-/ \alpha-$	0%
One parent HbH, 1 parent trait	$\alpha-/ \alpha-$	$\alpha\alpha/\alpha-$	0%
Both parents HbH	$\alpha-/ \alpha-$	$\alpha-/ \alpha-$	25%

HbH: hemoglobin H

Parents can also determine the likelihood of HbH disease in an offspring through genetic testing. However, because this is, in most cases, a mild condition, it is less likely to be considered information that is actionable in terms of altering reproductive decision making.^[20]

Section Summary: Clinical Utility

The clinical utility of genetic testing for α -thalassemia may occur in several settings. For confirming a diagnosis of α -thalassemia, because the diagnosis of clinically actionable types can generally be made on the basis of nongenetic testing, there is little utility to genetic testing. For patients with HbH disease, there may be a genotype-phenotype correlation for disease severity; however, no studies were identified that suggested patient management or outcomes would be altered by genetic testing. Therefore, genetic testing for determining the prognosis of HbH disease is not associated with improved clinical utility. Preconception (carrier) testing is likely to have clinical utility by providing incremental diagnostic information over biochemical testing. Genetic testing can identify the pattern of abnormal α -globin genes and estimate more precisely the risk of hydrops fetalis.

SUMMARY OF EVIDENCE

For individuals who have suspected α -thalassemia who receive genetic testing for α -thalassemia, the evidence includes case reports and case series documenting the association between pathogenic variants and clinical syndromes. Relevant outcomes are overall survival, disease-specific survival, test accuracy and validity, symptoms, and quality of life. For the α -thalassemia syndromes that have clinical implications, diagnosis can be made based on biochemical testing without genetic testing. The evidence is sufficient to determine that the technology is unlikely to improve the net health outcome.

For individuals who have hemoglobin H disease (α -thalassemia intermedia) who receive genetic testing for α -thalassemia, the evidence includes case series that correlate specific variants with prognosis of disease. Relevant outcomes are overall survival, disease-specific survival, symptoms, and quality of life. There is some evidence for a genotype-phenotype correlation with disease severity, but no current evidence indicates that patient management or outcomes would be altered by genetic testing. The evidence is insufficient to determine the effects of the technology on health outcomes.

For individuals who have biochemical evidence of α -thalassemia who are considering conception who receive genetic testing for α -thalassemia, the evidence includes case reports and case series that correlate pathogenic variants with clinical disease. Relevant outcomes are test accuracy, test validity, and changes in reproductive decision making. Preconception carrier testing is intended to avoid the most serious form of α -thalassemia, hemoglobin Bart's. This condition leads to intrauterine death or death shortly after birth, and is associated with increased obstetrical risks for the mother. Screening of populations at risk is first done by biochemical tests, including hemoglobin electrophoresis and complete blood count and peripheral smear, but these tests cannot reliably distinguish between the carrier and trait syndromes, and cannot determine which configuration of variants is present in α -thalassemia trait. They therefore cannot completely determine the risk of a pregnancy with hemoglobin Bart's and hydrops fetalis. Genetic testing can determine with certainty the number of abnormal genes present, and therefore can more precisely determine the risk of hydrops fetalis. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

PRACTICE GUIDELINE SUMMARY

The Society of Obstetricians and Gynaecologists of Canada published guidelines on carrier testing for thalassemia in 2008.^[20] These guidelines included the following recommendations:

1. Carrier screening for α -thalassemia should be offered to all woman from ethnic groups with an increased prevalence of α -thalassemia. Initial screening should consist of “complete blood count, hemoglobin electrophoresis or hemoglobin high performance liquid chromatography...” ferritin testing [and examination of peripheral] blood smear to identify H bodies.”
2. If a woman's screening is abnormal ..., then screening the partner should be performed [using the same battery of tests].”
3. “If both partners are found to be carriers of thalassemia ... or of a combination of thalassemia and a hemoglobin variant, they should be referred for genetic counseling.... Additional molecular studies may be required to clarify the carrier status of the parents and thus the risk to the fetus.”

SUMMARY

There is enough research to show that prenatal testing for α -thalassemia can improve health outcomes. Prenatal fetal testing informs reproductive decision making, including decisions regarding continuation of the pregnancy, birthing decisions, and enabling for timely treatment of a condition that could be treated either in utero or immediately after birth. Therefore, prenatal testing for α -thalassemia may be considered medically necessary.

There is enough research to show that carrier (preconception) testing can improve health outcomes for patients that have evidence of a possible α -thalassemia gene variant. Carrier testing is intended to avoid the most serious form of α -thalassemia, hemoglobin Bart's. Genetic testing is more effective than biochemical tests for determining the risk of a pregnancy with disorder. Clinical guidelines also recommend genetic carrier testing when biochemical test results are positive for possible α -thalassemia variants. Therefore, preconception (carrier) testing for α -thalassemia in prospective parents may be considered

medically necessary when both reproductive partners have evidence of possible α -thalassemia based on biochemical testing.

There is enough research to show that diagnosis of α -thalassemia syndromes can be made based on biochemical testing without genetic testing. Therefore, genetic testing to confirm a diagnosis of α -thalassemia is considered not medically necessary.

There is not enough research to show that genetic testing for α -thalassemia can improve health outcomes for patients with any other conditions, including people who have hemoglobin H disease (α -thalassemia intermedia). In addition, there are no clinical guidelines based on research that recommend this testing. Therefore, genetic testing is considered investigational for patients with hemoglobin H disease or for other clinical situations.

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21. BlueCross BlueShield Association Medical Policy Reference Manual "Genetic Testing for α -Thalassemia." Policy No. 2.04.104

CODES

Codes	Number	Description
CPT	81257	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; common deletions or variant (eg, Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5, Constant Spring)
	81258	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; known familial variant
	81259	;full gene sequence
	81269	;duplication/deletion variants
	81404	Molecular pathology procedure level 5
HCPCS	None	

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