

Genetic Testing for Alpha- and Beta- Thalassemia

Policy Number: AHS – M2131 – Genetic Testing for Alpha- and Beta-Thalassemia	Prior Policy Name and Number, as applicable: <ul style="list-style-type: none"> AHS-M2131 Genetic Testing for Alpha Thalassemia
Initial Presentation Date: 05/23/2016 Revision Date: 06/02/2021	

I. Policy Description

Alpha-thalassemia is characterized by impaired production of the alpha globin chains of hemoglobin, leading to a relative excess of gamma globin chains (fetus and newborn), or excess beta globin chains (children and adults) mainly due to deletion or mutation of the alpha globin genes. There are four alpha thalassemia syndromes, reflecting the loss of function of one, two, three, or all four of these alpha chain genes varying in severity from non-symptomatic to incompatibility with extrauterine life (Benz Jr & Angelucci, 2020; Martin & Thompson, 2013).

Beta-thalassemia is similarly characterized by impaired production of hemoglobin components but affects the beta chains instead of the alpha chains. This creates excess alpha globin chains, leading to hemolytic anemia, impaired iron handling, and other clinical symptoms (Benz Jr, 2021).

II. Related Policies

Policy Number	Policy Title
AHS-M2145	General Genetic Testing, Germline Disorders
AHS-M2146	General Genetic Testing, Somatic Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. If there is a conflict between this Policy and any relevant, applicable government policy [e.g. Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare] for a particular member, then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit their search website <http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx> or [the manual website](#)

1. Genetic counseling for alpha- or beta-thalassemia genetic testing **MEETS COVERAGE CRITERIA** and is recommended.

2. Preconception (carrier) testing for alpha- or beta-thalassemia in prospective parents **MEETS COVERAGE CRITERIA** when either parent has evidence of possible alpha-thalassemia (including alpha thalassemia minor, hemoglobin H disease [alpha thalassemia intermedia], or alpha thalassemia major) or beta-thalassemia (including beta thalassemia minor, beta thalassemia intermedia, or beta thalassemia major) based on biochemical testing.
3. Genetic testing to confirm a diagnosis of alpha- or beta- thalassemia **MEETS COVERAGE CRITERIA** when one of the parents is a known carrier or when other testing to diagnose cause of microcytic anemia has been inconclusive.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

4. Genetic testing for alpha- or beta-thalassemia in other clinical situations (recognizing that prenatal and newborn testing is not addressed in this policy) **DOES NOT MEET COVERAGE CRITERIA.**

IV. Scientific Background

Thalassemias result from deficiencies in hemoglobin biosynthesis due to mutations in or near the two globin gene clusters which encode the globin polypeptide subunits of hemoglobin (Benz Jr & Angelucci, 2020). Normal hemoglobin is a heterotetramer of two alpha globin chains and two beta globin chains (hemoglobin A) or two gamma globin chains (hemoglobin F). Well over 100 mutations have been documented to affect the biosynthesis or post-translational stability of the globin subunits needed for successful production of the large amounts of Hb needed for normal red cell homeostasis. Globin chain synthesis is very tightly controlled, such that the ratio of production of alpha to non-alpha chains is almost exactly 1:1 (Benz Jr, 2020).

Alpha thalassemia refers to thalassemias that result from impaired or absent production of alpha globin, leading to a relative excess of gamma globin (fetus and newborn), or excess beta globin (children and adults). Excess beta globin chains can form soluble homotetramers, but they are nonfunctional and unstable. This may lead to increased hemolysis and a variety of clinical manifestations, such as anemia, thrombosis, and skeletal changes. A diagnosis of alpha thalassemia is often confirmed by genetic testing, as assessment of the hemoglobin gene is inexpensive and convenient (Benz Jr, 2020).

The clinical severity is directly attributable to the net deficit of alpha globin synthesis but is complicated by the number of alpha globin genes affected, which of the two alpha globin loci is affected, and the degree to which the mutation blocks gene expression. In addition, combinations of defects in both alpha and beta globulins can balance each other out. Thus, understanding the broad spectrum of clinical severity in alpha thalassemia requires a detailed knowledge of the underlying genetic defect and the impact of these defects on the overall levels and balance of globin chain synthesis (Benz Jr, 2021).

The majority of cases of alpha thalassemia are attributable to deletion of alpha globin alleles, especially in Asia and Africa (Steinberg, 1999). However, more detailed analysis of globin gene

sequences suggests that some fairly common forms of alpha thalassemia that appear to arise from a deletion of one copy of an alpha globin gene are actually due to unequal crossover and recombination events that fuse the two alpha globin genes together into one (Benz Jr, 2020). Additionally, non-deletion alleles are also common, especially in the Mediterranean area, which contain mutations producing highly unstable alpha globin variants unable to produce intact hemoglobin (Benz Jr & Angelucci, 2020). Current research continues to identify novel mutations and improve thalassemia screening (He et al., 2018).

Beta-thalassemia is similar to alpha-thalassemia, with the beta chains of hemoglobin affected instead of the alpha chains. However, excess alpha globin chains do not form soluble homotetramers, causing them to aggregate when they accumulate in erythroid precursors. This causes clinical symptoms to be more severe, although the symptoms themselves are similar to alpha-thalassemia (anemia, iron overload, and so on) (Benz Jr, 2021; Benz Jr & Angelucci, 2020). There are two beta globin genes compared to four for the alpha chain. As with alpha-thalassemia, the severity of clinical presentation depends on the genotype of the beta globin genes (i.e. the ratio of beta to alpha globin chains). Mutations may result in a reduced expression (β^+) or absent expression (β^0). β^0 phenotypes are generally transfusion-dependent as they produce very little (if any) adult hemoglobin (Benz Jr, 2021).

Due to the frequency of thalassemias worldwide, carrier screening may be useful, particularly in areas such as Southeast Asia, Africa, and the Indian subcontinent. Both primary thalassemias are autosomal recessive genetic disorders so parents who are heterozygous carriers would have a 25% chance to have an affected child despite being asymptomatic themselves. Identification of an affected fetus could alter decisions during the pregnancy (Yates, 2021).

Below is a table summarizing the clinical genotypes and phenotypes of both thalassemia syndromes (Benz Jr, 2018; Benz Jr & Angelucci, 2020; Steinberg, 2019) (figure from Benz Jr).

Severity	Genotype	Anemia	Hemoglobin Analysis
Alpha Thalassemias			
Silent carrier	$\alpha\alpha / \alpha-$	None	Normal, <3% Hb Barts (gamma globin tetramer) at birth
Minor	$\alpha\alpha / --$ or $\alpha- / \alpha-$	Mild Microcytic	Normal, 3 to 8% Hb Barts at birth
Hb H disease	$\alpha- / --$	Moderate Microcytic	up to 30% HbH (beta globin tetramer), present in adults, up to 4% HbA ₂ (alpha and delta globin)
Major (fetal hydrops)	$-- / --$	Severe Microcytic, usually fatal	Hb Barts, Hb Portland (zeta and gamma globin), and HbH present, HbA, HbF, and HbA ₂ absent
Beta Thalassemias			

Minor (trait or carrier)	β / β^0 or β / β^+	Mild Microcytic	HbA ₂ (4% or more); HbF (up to 5%)
Intermedia (non-transfusion-dependent)	β^+ / β^+	Moderate Microcytic	HbA ₂ (4% or more); HbF (up to 50%)
Major (transfusion-dependent)	β^0 / β^0 or β^0 / β^+	Severe microcytic with target cells (typical Hb 3 to 4 g/dL)	HbA ₂ (5% or more); HbF (up to 95%); no HbA

Clinical Validity and Utility

He et al. (2017) examined a next-generation sequencing (NGS) panel's utility for thalassemia screening in Southwestern China. 951 individuals were tested, and the NGS screen found 471 carriers (49.5%) of thalassemia. In comparison, traditional methods (defined as "red cell indexes and hemoglobin electrophoresis, then DNA sequencing") identified only 209 carriers (22%) of thalassemia, missing 217 alpha-thalassemia carriers and 47 beta-thalassemia carriers (He et al., 2017). In a separate study by Zhang et al. (2019), because of studying 3,973 subjects that underwent hematological examinations and additional NGS and Gap-PCR due to being suspected thalassemia carriers, the researchers found that "approximately 2.88% thalassemia carriers would be missed by traditional genetic analysis. In addition, four novel thalassemia mutations and one novel abnormal hemoglobin mutation were identified." This research further corroborated the increased effectiveness of using NGS in screening thalassemia in an area of high disease prevalence (Zhang et al., 2019).

Nosheen et al. (2015) evaluated a preliminary screening program for beta-thalassemia. The screening program focused on families of beta-thalassemia major children. 98 samples were taken, and 57 were found to have a beta-thalassemia trait with elevated hemoglobin alpha 2. The mean hemoglobin alpha 2 level of the carriers was $5.2 \pm 0.56\%$ compared to $2.34 \pm 0.57\%$ in normal subjects. The authors suggested that screening programs and counseling for carriers could decrease incidence of beta-thalassemia major (Nosheen et al., 2015).

Satirapod et al. (2019) evaluated the clinical outcomes of using preimplantation genetic testing (PGT) in couples at risk of passing on beta thalassemia. Two components of PGT were used, PGT for monogenic disease (used for diagnosis) and PGT for aneuploidy (intended to identify chromosomal aberrations) A total of 15 couples were included and a total of 106 embryos were tested. After preimplantation testing, 12 of 15 women were able to obtain satisfactory genetic testing results (defined as non-disease affected embryos without chromosomal aberration and transfer within first two cycles). Of these, 9 women had successful implantations and 8 women had successful pregnancies with live births (deemed a 53.33% success rate). PGT assessment of genetic status was confirmed by pre- and post-natal genetic testing. Overall, the authors concluded that combined PGT-A and PGT-M was a useful technology to prevent beta-thalassemia in the offspring of recessive carriers. To increase the diagnostic efficiency of PGT-M, multiple displacement amplification (MDA) may be utilized as the first step. This conclusion was drawn by Fu, Shen, Chen, Wang, and Zhou (2019), who found in a

retrospective cohort study, that from 2,315 embryos tested, MDA yielded a 96.99% diagnostic efficiency, versus a PCR group, which only yielded 88.15%. MDA also enabled statistically significantly more embryos to be available for transfer as well when compared to the PCR group (74.28% vs 64.28%, respectively, $P < 0.001$) (Fu et al., 2019).

Chen et al. (2020) also conducted a similar study that evaluated “the efficacy of preimplantation genetic testing (PGT) for α - and β -double thalassemia combined with aneuploidy screening using next-generation sequencing (NGS).” From 12 couples that each carried both α - and β - thalassemia mutations, the researchers were able to facilitate 11 healthy live births from examining 112 embryos. This NGS-based SNP (single nucleotide polymorphism) haplotyping was demonstrated to “reduce misdiagnosis by linkage analyses with multiple SNP loci” and increase the number of diagnosis results, including those from detecting aneuploidy and identified mutation sites, in a single PGT cycle. It was also found that NGS-based SNP haplotyping could be performed “through directly detecting mutation sites with NGS and using affected embryos or gametes as probands.” This procedure benefits in eliminating multiple biopsies as well (Chen et al., 2020).

Shook, Haygood, and Quinn (2020) evaluated the accuracy of a specific pattern in hemoglobin separation tests. The authors desired to find if an “FSA” pattern corresponded to a final diagnosis of the sickle cell trait (HbAS), or a final diagnosis of sickle beta-thalassemia (HbS β^+). Traditionally, the FSA pattern has indicated a diagnosis of HbS β^+ ; however, the authors hypothesized that the FSA pattern truly indicates a diagnosis of HbAS instead. 31 newborns with an initial screening result of the FSA pattern (a suspected diagnosis of HbS β^+) were included. 30 of these newborns underwent protein-base confirmatory testing and 17 underwent confirmatory genetic testing. Of the newborns undergoing protein confirmatory testing, 23 had an “FSA” pattern, establishing a diagnosis of HbAS. Of the 8 remaining newborns with an FSA pattern, 7 underwent genetic testing which identified HbAS as well. Genetic testing also confirmed positive HbAS results in 10 newborns that tested initially positive by protein testing. The authors concluded that genetic testing had utility in newborn screening for hemoglobinopathies.

V. Guidelines and Recommendations

Canadian College of Medical Geneticists (CCMG) and Society of Obstetricians and Gynaecologists of Canada (SOGC) (Wilson et al., 2016)

The CCMG and SOGC published a joint guideline titled “Joint SOGC–CCMG Opinion for Reproductive Genetic Carrier Screening: An Update for All Canadian Providers of Maternity and Reproductive Healthcare in the Era of Direct-to-Consumer Testing” in 2016. Their recommendations addressing thalassemias/hemoglobinopathies are listed below:

- "Carrier screening for hemoglobinopathies should be offered to women/families from ethnic backgrounds with a reported increased carrier frequency, when red blood cell indices reveal a mean cellular volume < 80 fl, or electrophoresis reveals an abnormal hemoglobin type. However, the use of ethnicity alone in the carrier risk identification process may create screening inconsistency and missed opportunity for carrier identification, with both obstetrical and fetal implications. High clinical suspicion is required as well. Screening should be done in the pre-conception period or as early into the pregnancy as possible. (II-2A) (GRADE moderate/moderate)"

- “Carrier screening for thalassemia/hemoglobinopathies should be offered by the most responsible health care provider or reproductive genetic provider and include: complete blood count, hemoglobin (Hb) electrophoresis (HE) or Hb high performance liquid chromatography (HHPLC), quantification of Hb alpha 2 and fetal Hb, and serum ferritin/H bodies (blood smear stain using brilliant cresyl blue) if microcytosis (mean cellular volume < 80 fl) and/or hypochromia (mean cellular Hb < 27 pg) in the presence of a normal HE or HHPLC assessment. (II-2A) (GRADE moderate/moderate)”
- “If the female thalassemia screening results are abnormal, a hemoglobinopathy screening protocol should be undertaken for the male partner. (III-A) (GRADE low/moderate)”
- “If both reproductive partners are found to be carriers of thalassemia or a combination of thalassemia and hemoglobin variant, they should be referred for formal genetic counselling (reproductive risks, recommended prenatal testing, and diagnostic management). (II-3A) (GRADE moderate/moderate)” (Wilson et al., 2016)

The Thalassemia Longitudinal Cohort (Tubman et al., 2015)

The report on the Thalassemia Longitudinal Cohort (Tubman et al., 2015) recommends: “Obtaining genotyping to confirm the diagnosis and HLA typing for transplant evaluation for all patients who require chronic transfusion is strongly recommended. For pediatric patients, annual comprehensive follow up should include assessment of the availability of a related donor as well as a recommendation to bank cord blood and obtain HLA typing on all subsequently born full siblings.”

American College of Obstetrics and Gynecology (ACOG) (ACOG, 2007, 2017, 2018)

The ACOG Committee Opinion #691 (“Carrier Screening for Genetic Conditions”) states that: “Couples at risk of having a child with a hemoglobinopathy may benefit from genetic counseling to review their risk, the natural history of these disorders, prospects for treatment and cure, availability of prenatal genetic testing, and reproductive options. Prenatal diagnostic testing for the mutation responsible for sickle cell disease is widely available. Testing for α -thalassemia and β -thalassemia is possible if the mutations and deletions have been previously identified in both parents. These DNA-based tests can be performed using chorionic villi obtained by chorionic villus sampling or using cultured amniotic fluid cells obtained by amniocentesis. For some couples, preimplantation genetic diagnosis in combination with in vitro fertilization may be a desirable alternative to avoid termination of an affected pregnancy. Preimplantation genetic diagnosis has been successfully performed for sickle cell disease and most types of β -thalassemia” (ACOG, 2017). This was reaffirmed in 2020.

In 2018, ACOG reaffirmed its practice bulletin regarding hemoglobinopathies in pregnancy (originally published in 2007) (ACOG, 2018). The following recommendations are considered “Level A” and based on “good and consistent scientific evidence”.

- “Individuals of African, Southeast Asian, and Mediterranean descent are at increased risk for being carriers of hemoglobinopathies and should be offered carrier screening and, if both parents are determined to be carriers, genetic counseling.”
- “A complete blood count and hemoglobin electrophoresis are appropriate laboratory tests for screening for hemoglobinopathies. Solubility tests alone are inadequate for screening because they fail to identify important transmissible hemoglobin gene abnormalities affecting fetal outcome.”

- “Couples at risk for having a child with sickle cell anemia or thalassemia should be offered genetic counseling to review prenatal testing and reproduction options. Prenatal diagnosis of hemoglobinopathies is best accomplished by DNA analysis of cultured amniocytes or chorionic villi.”

ACOG also writes that “if the MCV [mean corpuscular volume] is below normal, iron deficiency anemia has been excluded, and the hemoglobin electrophoresis is not consistent with β -thalassemia trait (ie, there is no elevation of Hb A₂ or Hb F), then DNA-based testing should be used to detect α -globin gene deletions characteristic of α -thalassemia”. ACOG remarks that neither hemoglobin electrophoresis nor solubility testing can identify individuals with the α -thalassemia trait, only molecular genetic testing can (ACOG, 2007).

The Association of Public Health Laboratories (APHL) (APHL, 2015)

Molecular testing (APHL, 2015) can be added to resolve cases when the newborn has been transfused with packed red blood cells. Since the newborn’s phenotype is masked by the donor, DNA testing can be used to identify any abnormal hemoglobins.

National Health Service (NHS, 2017)

NHS states that first, the mean cell hemoglobin (MCH) must be measured in a screening for thalassemia. The NHS presents this decision tree of when to test for thalassemia:

“1. Is the MCH <25pg?

2. Is the woman’s family origin identified as high risk from the Family Origin Questionnaire: China (including Hong Kong), Southeast Asia (especially Thailand, Taiwan, Cambodia, Laos, Vietnam, Burma, Malaysia, Singapore, Indonesia or Philippines), Cyprus, Greece, Sardinia, Turkey, or unknown?

If the answer to both questions is yes, testing of the baby’s biological father must be offered if he is also from a high-risk area or unknown.

If the baby’s biological father is suspected of having α 0 thalassaemia, the samples on both biological parents must be sent for DNA analysis for α 0 thalassaemia mutations.

If one biological parent is a suspected carrier of α 0 thalassaemia and the other is a carrier of a thalassaemia and is also from one of the high-risk groups for α 0 thalassaemia with an MCH < 25pg, both biological parents should be screened for α 0 thalassaemia by DNA analysis.”

The NHS states that 99% of α 0 thalassemia cases have an MCH of < 25 pg. However, the NHS acknowledges that this algorithm may miss some beta-thalassemia carriers (NHS, 2017).

Public Health England (PHE) (PHE, 2021)

The PHE highlights the importance of antenatal screening. If the baby’s mother is identified as a carrier, the biological father should also be tested. Both prenatal diagnosis and genetic counselling are recommended by the PHE (PHE, 2021).

British Society for Haematology (BSH) (Ryan et al., 2010)

The BSH provides the following recommendations:

- “Antenatal screening/testing of pregnant women should be carried out according to the guidelines of the NHS Sickle Cell and Thalassaemia Screening programme.
- Laboratories performing antenatal screening should utilize methods capable of detecting significant variants and be capable of quantitating haemoglobins A₂ and F at the cut-off points required by the national antenatal screening program (Ryan et al., 2010).”

Genetic counseling is also permitted for prospective parents.

VI. State and Federal Regulations, as applicable

A. Food and Drug Administration (FDA)

A search on the FDA website of “thalassemia” on April 15, 2021 did not yield any genetic testing results (FDA, 2021). Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

B. Centers for Medicare & Medicaid Services (CMS)

A53493 Billing and Coding: MolDX: HBB Gene Tests - <https://www.cms.gov/medicare-coverage-database/view/article.aspx?articleid=53493&ver=18&bc=0>

A56853 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT) - <https://www.cms.gov/medicare-coverage-database/view/article.aspx?articleid=56853&ver=31&Date=&DocID=A56853&bc=gQAAAAIAAAA&bc=0>

L35025 MolDX: Molecular Diagnostic Tests (MDT) - <https://www.cms.gov/medicare-coverage-database/view/lcd.aspx?lcdid=35025&ver=91&bc=0>

VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
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	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; full gene sequence
81269	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; duplication/deletion variants
81361	HBB (hemoglobin, subunit beta) (eg, sickle cell anemia, beta thalassemia, hemoglobinopathy); common variant(s) (eg, HbS, HbC, HbE)
81362	HBB (hemoglobin, subunit beta) (eg, sickle cell anemia, beta thalassemia, hemoglobinopathy); known familial variant(s)
81363	HBB (hemoglobin, subunit beta) (eg, sickle cell anemia, beta thalassemia, hemoglobinopathy); duplication/deletion variant(s)
81364	HBB (hemoglobin, subunit beta) (eg, sickle cell anemia, beta thalassemia, hemoglobinopathy); full gene sequence
S3845	Genetic testing for alpha-thalassemia
S3846	Genetic testing for hemoglobin E beta-thalassemia
96040	Medical genetics and genetic counseling services, each 30 minutes face-to-face with patient/family
S0265	Genetic counseling, under physician supervision, each 15 minutes

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Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

VIII. Evidence-based Scientific References

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