Genetic Testing for Alpha- and Beta- Thalassemia

Policy Number: AHS – M2131 – Genetic Testing for Alpha- and Beta-Thalassemia

Initial Presentation Date: 1/01/2020
Revision Date: 1/01/2020

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, 2018b; 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 (Schrier, 2018).

Related Policies

<table>
<thead>
<tr>
<th>Policy Number</th>
<th>Policy Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHS-M2145</td>
<td>General Genetic Testing, Germline Disorders</td>
</tr>
<tr>
<td>AHS-M2146</td>
<td>General Genetic Testing, Somatic Disorders</td>
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</tbody>
</table>

Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request

1. 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.

2. 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.
3. Genetic testing for alpha- or beta-thalassemia in other clinical situations (recognizing that prenatal testing is not addressed in this policy) is **EXPERIMENTAL AND INVESTIGATIONAL**.

**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, 2018b). 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, 2018c).

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, 2018c).

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 (Schrier, 2017).

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, 2018c). 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, 2018b). Current research continues to identify novel mutations and improve thalassemia screening (S. 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, 2018b; Schrier, 2017, 2018). 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 ($\beta^+$) or absent expression ($\beta^0$). $\beta^0$ phenotypes are generally transfusion-dependent as they produce very little (if any) adult hemoglobin (Schrier, 2018).

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

<table>
<thead>
<tr>
<th>Severity</th>
<th>Genotype</th>
<th>Anemia</th>
<th>Hemoglobin Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alpha Thalassemias</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silent carrier</td>
<td>a a / a -</td>
<td>None</td>
<td>Normal, &lt;3% Hb Barts (gamma globin tetramer) at birth</td>
</tr>
<tr>
<td>Minor</td>
<td>a a / - - or</td>
<td>Mild Microcytic</td>
<td>Normal, 3 to 8% Hb Barts at birth</td>
</tr>
<tr>
<td></td>
<td>a - / a -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb H disease</td>
<td>a - / - -</td>
<td>Moderate Microcytic</td>
<td>up to 30% HbH (beta globin tetramer), present in adults, up to 4% HbA₂ (alpha and delta globin)</td>
</tr>
<tr>
<td>Major (fetal hydrops)</td>
<td>- - / - -</td>
<td>Severe Microcytic, usually fatal</td>
<td>Hb Barts, Hb Portland (zeta and gamma globin), and HbH present, HbA, HbF, and HbA₂ absent</td>
</tr>
</tbody>
</table>

| **Beta Thalassemias**                           |
| Minor (trait or carrier) | β / β⁰ or β / β⁺ | Mild Microcytic | HbA₂ (4% or more); HbF (up to 5%)                                                   |
| Intermedia (non-transfusion-dependent) | β⁺ / β⁺ | Moderate Microcytic | HbA₂ (4% or more); HbF (up to 50%)                                                 |
| Major (transfusion-dependent)     | β⁰⁺ / β⁺ or β⁰⁺ / β⁺ | Severe microcytic with target cells (typical Hb 3 to 4 g/dL) | HbA₂ (5% or more); HbF (up to 95%); no HbA                                           |

**Clinical Validity and Utility**

He et al 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 (J. He et al., 2017).

Nosheen et al 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±0.56% compared to 2.34±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).

**Guidelines and Recommendations**

The Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists (CCMG) and the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada
(SOGC) published guidelines on carrier testing for thalassemia in 2008. The guidelines included the following recommendations (Langlois et al., 2008):

- “Carrier screening for thalassemia and hemoglobinopathies should be offered to a woman if she and/or her partner are identified as belonging to an ethnic population whose members are at higher risk of being carriers. Ideally, this screening should be done pre-conceptionally or as early as possible in the pregnancy.”
- “If both partners are found to be carriers of thalassemia or an Hb variant, or of a combination of thalassemia and a hemoglobin variant, they should be referred for genetic counselling. Ideally, this should be prior to conception, or as early as possible in the pregnancy. Additional molecular studies may be required to clarify the carrier status of the parents and thus the risk to the fetus.”
- “Prenatal diagnosis should be offered to the pregnant woman/couple at risk for having a fetus affected with a clinically significant thalassemia or hemoglobinopathy. Prenatal diagnosis should be performed with the patient's informed consent. If prenatal diagnosis is declined, testing of the child should be done to allow early diagnosis and referral to a pediatric hematology centre, if indicated.”

**The Thalassemia Longitudinal Cohort**

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)**

The ACOG Committee Opinion #691 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, 2018).

**The Association of Public Health Laboratories (APHL)**

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 a0 thalassaemia, the samples on both biological parents must be sent for DNA analysis for a0 thalassaemia mutations.

If one biological parent is a suspected carrier of a0 thalassaemia and the other is a carrier of a thalassaemia and is also from one of the high-risk groups for a0 thalassaemia with an MCH < 25pg, both biological parents should be screened for a0 thalassaemia by DNA analysis.”

The NHS states that 99% of a0 thalassemia cases have an MCH of < 25 pg. However, the NHS acknowledges that this algorithm may miss some beta-thalassemia carriers (NHS, 2017).


These joint guidelines include statements on thalassemia / hemoglobinopathies. Their recommendations are as follows:

- “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.”
- “Carrier screening for thalassemia/hemoglobinopathies should be offered by the most responsible health care provider or reproductive genetic provider and include: a 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.”
- “If the female thalassemia screening results are abnormal, a hemoglobinopathy screening protocol should be undertaken for the male partner.”
- “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) (Wilson et al., 2016)”.

State and Federal Regulations, as applicable

A search on the FDA website of “thalassemia” on May 17, 2019, did not yield any genetic testing results. 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.
### Applicable CPT/HCPCS Procedure Codes

<table>
<thead>
<tr>
<th>Code Number</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81257</td>
<td>HBA1/HBA2 (alpha globin 1 and alpha globin 2) (e.g., alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis, for common deletions or variant (e.g., Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5 and Constant Spring)</td>
</tr>
<tr>
<td>81258</td>
<td>HBA1/HBA2 (alpha globin 1 and alpha globin 2) (e.g., alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; known familial variant</td>
</tr>
<tr>
<td>81259</td>
<td>HBA1/HBA2 (alpha globin 1 and alpha globin 2) (e.g., alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; full gene sequence</td>
</tr>
<tr>
<td>81269</td>
<td>HBA1/HBA2 (alpha globin 1 and alpha globin 2) (e.g., alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; duplication/deletion variants</td>
</tr>
<tr>
<td>S3845</td>
<td>Genetic testing for alpha-thalassemia</td>
</tr>
<tr>
<td>S3846</td>
<td>Genetic testing for hemoglobin E beta-thalassemia</td>
</tr>
</tbody>
</table>


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

### Evidence-based Scientific References


Benz, E. (2018a). Classical thalassemia syndromes (genotypes and laboratory findings). In *UpToDate*. Waltham. MA.


**Policy Implementation/Update Information**

1/1/20 New Policy

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