

Corporate Medical Policy

Genetic Testing for Alpha- and Beta- Thalassemia AHS – M2131

File Name: genetic_testing_for_alpha_and_beta_thalassemia
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Description of Procedure or Service

Alpha-thalassemia is characterized by an 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, 2023; 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, 2023).

When pursuing genetic testing for alpha- or beta-thalassemia, genetic counseling is strongly recommended.

For guidance on prenatal screening and preconception screening for alpha- or beta-thalassemia, please see AHS-M2179-Prenatal Screening (Genetic).

Related Policies

General Genetic Testing, Germline Disorders AHS-M2145

General Genetic Testing, Somatic Disorders AHS-M2146

******Note: This Medical Policy is complex and technical. For questions concerning the technical language and/or specific clinical indications for its use, please consult your physician.***

Policy

BCBSNC will provide coverage for genetic testing for alpha- and beta- thalassemia when it is determined the medical criteria or reimbursement guidelines below are met.

Benefits Application

This medical policy relates only to the services or supplies described herein. Please refer to the Member's Benefit Booklet for availability of benefits. Member's benefits may vary according to benefit design; therefore member benefit language should be reviewed before applying the terms of this medical policy.

When Genetic Testing for Alpha- and Beta- Thalassemia is covered

Reimbursement for genetic testing to confirm an alpha- or beta-thalassemia diagnosis is allowed in **any** of the following situations:

- a. For individuals for whom one parent is a known carrier of alpha- or beta-thalassemia.

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- b. For individuals for whom other testing to diagnose the cause of microcytic anemia has been inconclusive

When Genetic Testing for Alpha- and Beta- Thalassemia is not covered

For all other situations not described above, genetic testing for alpha- or beta- thalassemia is considered investigational.

Policy Guidelines

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, 2023). 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, 2023b).

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, 2023b).

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, 2023c).

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, 2023c). 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, 2023). 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, 2023c; Benz Jr & Angelucci, 2023). 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, 2023c).

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

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child despite being asymptomatic themselves. Identification of an affected fetus could alter decisions during the pregnancy (Yates, 2023).

Below is a table summarizing the clinical genotypes and phenotypes of both thalassemia syndromes (Benz Jr, 2013a; Benz Jr & Angelucci, 2023; Steinberg, 2023) (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	Mild Microcytic	Normal, 3 to 8% Hb Barts at birth
	$\alpha- / \alpha-$		
Hb H disease	$\alpha- / --$	Moderate Microcytic	Up to 30% HbH (beta globin tetramer), present in adults, up to 4% HbA2 (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 HbA2 absent
Beta Thalassemias			
Minor (trait or carrier)	β / β^0 or $\beta / \beta+$	Mild Microcytic	HbA2 (4% or more); HbF (up to 5%)
Intermedia (non-transfusion-dependent)	$\beta+ / \beta+$	Moderate Microcytic	HbA2 (4% or more); HbF (up to 50%)
Major (transfusion-dependent)	β^0 / β^0 or $\beta^0 / \beta+$	Severe microcytic with target cells (typical Hb 3 to 4g/dL)	HbA2 (5% or more); HbF (up to 95%); no HbA

Analytical Validity

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

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corroborated the increased effectiveness of using NGS in screening thalassemia in an area of high disease prevalence (Zhang et al., 2019).

Shook et al. (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 (HbSβ⁺). Traditionally, the FSA pattern has indicated a diagnosis of HbSβ⁺; 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 HbSβ⁺) 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 (Shook et al., 2020).

Chen et al. (2021) established an effective NGS protocol for four-factor preimplantation genetic testing (PGT) to diagnose α- and β-thalassemia. Three couples, in whom both partners were α- and β-double thalassemia carriers, underwent PGT and a total of 35 biopsied trophectoderm samples underwent multiple displacement amplification (MDA). Using NGS-based single-nucleotide polymorphism (SNP) haplotyping, these samples were analyzed. “51.5% (17/33) of the embryos were diagnosed as unaffected non-carriers or carriers. Of the 17 unaffected embryos, nine (52.9%) were tested further and identified as euploid via NGS-based aneuploid screening, in which five had HLA types matching affected children.” The authors conclude that NGS-SNP was effective in performing PGT for multipurpose detection (Chen et al., 2021).

Clinical Utility and Validity

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±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).

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 et al. (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

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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).

Guidelines and Recommendations

American College of Medical Genetics and Genomics (ACMG)

In 2021, ACMG released an updated guideline for screening for autosomal recessive and X-linked conditions during pregnancy and preconception. Their practice resource aims to recommend “a consistent and equitable approach for offering carrier screening to all individuals during pregnancy and preconception” and replaces any earlier ACMG position statements on prenatal/preconception expanded carrier screening and provide the following recommendations:

- “Carrier screening enables those screened to consider their reproductive risks, reproductive options, and to make informed decisions.”
- “The phrase “expanded carrier screening” be replaced by “carrier screening.”
- “Adopting a more precise tiered system based on carrier frequency:
 - o Tier 4: $<1/200$ carrier frequency (includes Tier 3) genes/condition will vary by lab
 - o Tier 3: $\geq 1/200$ carrier frequency (includes Tier 2) includes X-linked conditions
 - o Tier 2: $\geq 1/100$ carrier frequency (includes Tier 1)
 - o Tier 1: CF [Cystic Fibrosis] + SMA [spinal muscular atrophy] + Risk Based Screening
 - “Tier 1 screening conveys the recommendations previously adopted by ACMG and ACOG” and “adopts an ethnic and population neutral approach when screening for cystic fibrosis and spinal muscular atrophy. Beyond these two conditions, additional carrier screening is determined after risk assessment, which incorporates personal medical and family history as well as laboratory and imaging information where appropriate”
 - “Tier 2 carrier screening stems from an ACOG recommendation for conditions that have a severe or moderate phenotype and a carrier frequency of at least $1/100$.” However, “data demonstrate that carrier screening for two common conditions using a carrier frequency threshold of $1/100$ may not be equitable across diverse populations. Others have shown that limiting the carrier frequency to $\geq 1/100$ creates missed opportunities to identify couples at risk for serious conditions.”
 - “We define Tier 3 screening as carrier screening for conditions with a carrier frequency $\geq 1/200$. . . Tier 2 and Tier 3 screening prioritize carrier frequency as a way to think about conditions most appropriate for screening in the general population. However, when ACOG proposed this level, they did not specify whether it was thinking about carrier frequency in terms of the global population or subpopulations. We use “carrier frequency” to mean in any ethnic group with reasonable representation in the United States.”
 - “Tier 4 includes genes less common than those in Tier 3 and can identify additional at-risk couples. Tier 4 has no lower limit carrier screening frequency and can greatly extend the number of conditions screened . . . the clinical validity at this level of carrier screening may be less compelling, therefore we suggest reserving this level of screening for consanguineous pregnancies (second cousins or closer) and in couples where family or medical history suggests Tier 4 screening might be beneficial . . . Importantly, patients should understand that their chance of being a carrier for one or more conditions increases as the number of conditions screened is increased.”
- “All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening.
- Tier 4 screening should be considered:
 - o When a pregnancy stems from a known or possible consanguineous relationship (second cousins or closer);

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- o When a family or personal medical history warrants.
- ACMG does NOT recommend:
 - o Offering Tier 1 and/or Tier 2 screening, because these do not provide equitable evaluation of all racial/ethnic groups.
 - o Routine offering of Tier 4 panels.
- “Carrier screening paradigms should be ethnic and population neutral and more inclusive of diverse populations to promote equity and inclusion.”
- “All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening for autosomal recessive [Table 1 & 5] . . . conditions.”
- “Reproductive partners of pregnant patients and those planning a pregnancy may be offered Tier 3 carrier screening for autosomal recessive conditions [Table 1 & 5] when carrier screening is performed simultaneously with their partner.”
- “When Tier 1 or Tier 2 carrier screening was performed in a prior pregnancy, Tier 3 screening should be offered” (Gregg et al., 2021).

Table 1. Autosomal recessive genes for screening with carrier frequency $\geq 1/50$.

OMIM gene	OMIM gene name	Maximum carrier frequency ^a	OMIM phenotype	Conditions
141900	<i>HBB</i>	0.119837	603903 613985	Sickle cell anemia β -thalassemia
613208	<i>XPC</i>	0.050885	278720	Xeroderma pigmentosum
606933	<i>TYR</i>	0.049337	203100 606952	Oculocutaneous albinism type 1A and 1B
613815	<i>CYP21A2</i>	0.048459	201910	Congenital adrenal hyperplasia due to 21-hydroxylase deficiency
612349	<i>PAH</i>	0.046068	261600	Phenylketonuria
602421	<i>CFTR</i>	0.040972	219700	Cystic fibrosis
600985	<i>TNXB</i>	0.035134	606408	Ehlers–Danlos-like syndrome due to tenascin-X deficiency
606869	<i>HEXA</i>	0.033146	272800	Tay–Sachs disease
121011	<i>GJB2</i>	0.026200	220290 601544	Nonsyndromic hearing loss recessive 1A Nonsyndromic hearing loss dominant 3A
602858	<i>DHCR7</i>	0.023709	270400	Smith–Lemli–Opitz syndrome
277900	<i>ATP7B</i>	0.021983	606882	Wilson disease
608034	<i>ASPA</i>	0.019856	271900	Canavan disease
607008	<i>ACADM</i>	0.016583	201450	Medium-chain acyl-coenzyme A dehydrogenase deficiency
602716	<i>NPHS1</i>	0.015994	256300	Finnish congenital nephrotic syndrome
601785	<i>PMM2</i>	0.015877	212065	Carbohydrate-deficient glycoprotein syndrome type Ia
607440	<i>FKTN</i>	0.015660	611615 253800	Cardiomyopathy, dilated, 1X Walker–Warburg congenital muscular dystrophy
605646	<i>SLC26A4</i>	0.015422	600791 274600	Deafness autosomal recessive 4 Pendred syndrome
126340	<i>ERCC2</i>	0.015255	610756 601675	Cerebroculofacioskeletal syndrome 2 Trichothiodystrophy 1, photosensitive
603297	<i>DYNC2H1</i>	0.014817	613091	Short-rib thoracic dysplasia 3 with or without polydactyly

OMIM Online Mendelian Inheritance in Man.⁵⁵
^aValues round to ≥ 0.02 (two decimal places).

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Table 5. Genes that were ascertained for screening outside of the gnomAD criteria^a.

OMIM gene	OMIM gene name	Published carrier frequency ^b	Rationale for inclusion	Ethnic group	OMIM phenotype	Conditions
141800	<i>HBA1</i>	U ^c	Carrier frequency	SEA and others	604131	α -Thalassemia
141850	<i>HBA2</i>	U ^c	Carrier frequency	SEA and others	604131	α -Thalassemia
600354	<i>SMN1</i>	1/60 ¹⁸	ACOG/ACMG and carrier frequency	US panethnic	253300 253550 253400 271150	Spinal muscular atrophy types: I, II, III, IV
604982	<i>HPS1</i>	1/59 ⁵⁶⁻⁵⁸	Carrier frequency	PR	203300	Hermansky Pudlak S. 1
606118	<i>HPS3</i>	1/59 ⁵⁶	Carrier frequency	PR	614072	Hermansky Pudlak S. 3
603722	<i>ELP1</i>	1/32 ⁵⁹	ACOG/ACMG and carrier frequency	AJ	223900	Familial dysautonomia
606829	<i>FXN</i>	1/60–1/100 ⁶⁰	Carrier frequency	Caucasians ^d	229300	Friedreich ataxia
238331	<i>DLD</i>	~1/100 ^{59,61}	Carrier frequency	AJ	246900	Dihydroliipoamide dehydrogenase deficiency
161650	<i>NEB</i>	1/168 ⁵⁹	Carrier frequency	AJ	256030	Nemaline myopathy 2
606397	<i>CLRN1</i>	1/120 ⁵⁹	Carrier frequency	AJ	276902	Usher syndrome 3a
604610	<i>BLM</i>	1/100 ⁵⁹	ACMG and carrier frequency	AJ	210900	Bloom syndrome

ACMG American College of Medical Genetics and Genomics, ACOG American College of Obstetricians and Gynecologists, AJ Ashkenazi Jewish ($\geq 2\%$ of the US population), OMIM Online Mendelian Inheritance in Man,⁵⁵ PR Puerto Rican, SEA South East Asian.
^aCarrier frequency of a sequence variant is $< 1/200$, if reported in gnomAD.⁵⁰
^bDiagnostic laboratory data was not used for carrier frequency data.
^cSpecific data for general US population not available; however, recognized as common among many US immigrant populations.⁶²
^dThis term is no longer used by the journal but is used in the original article to which these studies refer. We have therefore not changed the term but recognize it does not accurately describe the ancestry of the populations originally studied.⁴⁶

(Gregg et al., 2021)

Canadian College of Medical Geneticists (CCMG) and Society of Obstetricians and Gynaecologists of Canada (SOGC)

The CCMG and SOGC published a joint guideline titled “Carrier Screening for Thalassemia and Hemoglobinopathies in Canada” in 2008. Their recommendations addressing thalassemia’s/hemoglobinopathies are listed below:

1. “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. (II-2A)
2. Screening should consist of a complete blood count, as well as hemoglobin electrophoresis or hemoglobin high performance liquid chromatography. This investigation should include quantitation of HbA2 and HbF. In addition, if there is microcytosis (mean cellular volume < 80 fL) and/or hypochromia (mean cellular hemoglobin < 27 pg) in the presence of a normal hemoglobin electrophoresis or high-performance liquid chromatography the patient should be investigated with a brilliant cresyl blue stained blood smear to identify H bodies. A serum ferritin (to exclude iron deficiency anemia) should be performed simultaneously. (III-A)
3. If a woman’s initial screening is abnormal (e.g., showing microcytosis or hypochromia with or without an elevated HbA2, or a variant Hb on electrophoresis or high-performance liquid chromatography) then screening of the partner should be performed. This would include a complete blood count as well as hemoglobin electrophoresis or HPLC, HbA2 and HbF quantitation, and H body staining. (III-A)
4. 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

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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. (II-3A)

5. 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. (II-3A)

6. Prenatal diagnosis by DNA analysis can be performed using cells obtained by chorionic villus sampling or amniocentesis. Alternatively, for those who decline invasive testing and are at risk of hemoglobin Bart's hydrops fetalis (four-gene deletion α -thalassemia), serial detailed fetal ultrasound for assessment of the fetal cardiothoracic ratio (normal < 0.5) should be done in a centre that has experience conducting these assessments for early identification of an affected fetus. If an abnormality is detected, a referral to a tertiary care centre is recommended for further assessment and counselling. Confirmatory studies by DNA analysis of amniocytes should be done if a termination of pregnancy is being considered. (II-3A)

7. The finding of hydrops fetalis on ultrasound in the second or third trimester in women with an ethnic background that has an increased risk of α -thalassemia should prompt immediate investigation of the pregnant patient and her partner to determine their carrier status for α -thalassemia. (III-A)" (Langlois et al., 2008).

The Thalassemia Longitudinal Cohort

The report on the Thalassemia Longitudinal Cohort 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" (Tubman et al., 2015).

American College of Obstetrics and Gynecology (ACOG)

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."

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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).

National Health Service (NHS)

The NHS released standards for antenatal laboratories working with the NHS sickle cell and thalassaemia (SCT) screening program (NHS, 2021). The referral guidelines for antenatal screening specimens are as follows:

Biological mother carrier state	Biological father carrier state	Further studies by DNA analysis
No abnormalities detected	Testing of baby's biological father not required	None required
Any abnormal Hb or thalassaemia	No abnormality detected	None required
HbS	HbS or HbC	None required until PND
HbS	HbO ^{Arab} , D ^{Punjab} , E, Lepore, β thalassaemia or $\delta\beta$ thalassaemia	If PND is being considered send bloods for mutation confirmation, provided it does not limit options available
HbS	HPFH	Send bloods for mutation confirmation. PND is not usually indicated when HPFH has been confirmed
HbS + α thalassaemia	Assess risk as per HbS alone, unless family origins indicate a high risk of α^0 thalassaemia	Assess risk as per HbS alone, unless family origins indicate a high risk of α^0 thalassaemia
HbC	HbS	None required until PND
HbD	HbS	If PND is being considered send bloods for mutation confirmation, provided it does not limit options available
HbO ^{Arab}	HbS, β thalassaemia or $\delta\beta$ thalassaemia	If PND is being considered send bloods for mutation confirmation, provided it does not limit options available
Hb Lepore	HbS, E, O ^{Arab} , Lepore, β thalassaemia or $\delta\beta$ thalassaemia	If PND is being considered send bloods for mutation confirmation, provided it does not limit options available.
HbE	β thalassaemia, Hb Lepore, $\delta\beta$ thalassaemia, HbS	If PND is being considered send bloods for mutation confirmation, provided it does not limit options available
HbE	α thalassaemia (MCH < 25pg)	Send bloods for mutation confirmation if high risk family origins for α^0 thalassaemia, consider impact on options available
β or $\delta\beta$ thalassaemia	HbS, E, O ^{Arab} , Lepore, β thalassaemia or $\delta\beta$ thalassaemia	If PND is being considered send bloods for mutation confirmation, provided it does not limit options available
β or $\delta\beta$ thalassaemia	α thalassaemia (high risk family origins)	Send bloods for mutation confirmation if high risk family origins for α^0 thalassaemia, consider impact on options available
HPFH	HbS, E, O ^{Arab} , Lepore, β or $\delta\beta$ thalassaemia	Send bloods for mutation confirmation. PND is not usually indicated when HPFH has been confirmed
α thalassaemia (MCH < 27pg but \geq 25pg)	Testing of baby's biological father not required	None required
α thalassaemia (MCH < 25pg)		
1. Low risk α^0 thal family origins in either biological parent	Testing of baby's biological father not required	None required
2. High risk α^0 thal family origins in both biological parents or unknown	Test baby's biological father and if MCH < 25pg irrespective of any other phenotype detected	Send maternal and paternal bloods for mutation confirmation, consider impact on options available.

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The Association of Public Health Laboratories (APHL)

APHL states that “Molecular testing 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” (APHL, 2015).

Public Health England (PHE)

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, 2022).

British Society for Haematology (BSH)

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.

The Thalassemia International Foundation (TIF)

The TIF provided recommendations for the management of transfusion dependent Thalassemia. The following recommendations were made (TIF, 2021):

- “Molecular genetic testing is available in clinical laboratories and may be useful for predicting the clinical phenotype in some cases as well as enabling presymptomatic diagnosis of at-risk family members and prenatal diagnosis.
- Molecular analysis is not required to confirm the diagnosis of a β carrier, but it is necessary to confirm the α thalassemia carrier status (grade A)
- Since the prevalent pathogenic variants of the β globin gene are limited in each at-risk population, a PCR method designed to detect the common specific mutation simultaneously should be used initially (grade B)
- β globin gene sequence analysis may be considered first if the affected individual is not of an ancestry at high risk or if targeted analysis reveals only one or no pathogenic variant (grade B)
- α thalassemias are mainly due to deletions of different length and they can be detected preferentially by reverse dot blot and Gap-PCR (grade B)
- Methods that may be used to detect rare or unknown deletions include: Southern blotting (now fallen into abeyance), quantitative PCR, long-range PCR and, above all, MLPA (grade B)” (TIF, 2021).

State and Federal Regulations, as applicable

Food and Drug Administration (FDA)

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). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

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Billing/Coding/Physician Documentation Information

This policy may apply to the following codes. Inclusion of a code in this section does not guarantee that it will be reimbursed. For further information on reimbursement guidelines, please see Administrative Policies on the Blue Cross Blue Shield of North Carolina web site at www.bcbsnc.com. They are listed in the Category Search on the Medical Policy search page.

Applicable service codes: 81257, 81258, 81259, 81269, 81361, 81362, 81363, 81364, S3845, S3846,

BCBSNC may request medical records for determination of medical necessity. When medical records are requested, letters of support and/or explanation are often useful, but are not sufficient documentation unless all specific information needed to make a medical necessity determination is included.

Scientific Background and Reference Sources

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Policy Implementation/Update Information

For policy titled: Genetic Testing for Alpha Thalassemia

- 1/1/2019 BCBSNC will provide coverage for genetic testing for alpha thalassemia when it is determined to be medically necessary because the criteria and guidelines are met. Medical Director review 1/1/2019. (jd)
- 8/13/2019 Specialty Matched Consultant Advisory Panel review 7/2019. Medical Director review 7/2019. (jd)

For policy titled: Genetic Testing for Alpha- and Beta- Thalassemia:

- 9/10/2019 Reviewed by Avalon 2nd Quarter 2019 CAB with title change. Added characteristics of beta-thalassemia and Related Policies to the Description section. Indications for beta-thalassemia added to the policy statement along with both the When Covered and When

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Not Covered sections. Policy guidelines updated. Added the following codes to the Billing/Coding section: *S3845*, *S3846*, and removed code table. References updated. Medical Director review 8/2019. (jd)

- 10/29/19 Wording in the Policy, When Covered, and/or Not Covered section(s) changed from Medical Necessity to Reimbursement language, where needed. (hb)
- 7/28/20 Reviewed by Avalon 2nd Quarter 2020 CAB. Added item 1 reimbursement language for genetic counseling and revised item 3 from medically necessary to reimbursement language under the When Covered section. Policy guidelines and references updated. Added the following codes to the Billing/Coding section: 81361, 81362, 81363, 81364, 96040, S0265. Specialty Matched Consultant Advisory Panel review 7/2020. Medical Director review 7/2020. (jd)
- 8/24/21 Reviewed by Avalon 2nd Quarter 2021 CAB. Description, Policy guidelines, and Reference sections update. Specialty Matched Consultant Advisory Panel review 7/2021. Medical Director review 7/2021. (jd)
- 9/13/22 Reviewed by Avalon 2nd Quarter 2022 CAB. Description, Policy guidelines, and Reference sections updated. No change to policy statement. Medical Director review 7/2022. (tm)
- 8/15/23 Reviewed by Avalon 2nd Quarter 2023 CAB. Updated the background, guidelines and recommendations, and evidence-based scientific references. Genetic counseling recommendation moved from When Covered to the Policy Description. Medical Director review 7/2023. (rp)

Medical policy is not an authorization, certification, explanation of benefits or a contract. Benefits and eligibility are determined before medical guidelines and payment guidelines are applied. Benefits are determined by the group contract and subscriber certificate that is in effect at the time services are rendered. This document is solely provided for informational purposes only and is based on research of current medical literature and review of common medical practices in the treatment and diagnosis of disease. Medical practices and knowledge are constantly changing and BCBSNC reserves the right to review and revise its medical policies periodically.