Cleveland Clinic
Laboratories

Carrier Screen Test White Paper

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Background

In a joint statement, the American College of Obstetricians and Gynecologists, American College of Medical Genetics and Genomics, Perinatal Quality Foundation, National Society of Genetic Counselors and Society for Maternal-Fetal Medicine point out that carrier screening is an important part of prenatal care.[1,2] They recommend that healthcare providers offer patients the option of having carrier screening while educating them about the types of conditions covered by the test.

Carrier screening has historically been used in conjunction with ethnic background to identify individuals who may be at an increased risk to have a child with a genetic disorder found commonly in that ethnic group. It is becoming increasingly difficult for many people to identify a single ethnic group, due to admixture or because full knowledge of family history is unknown or not available. Expanded carrier screening has grown from this difficulty in assigning ethnicity.

Carrier screens primarily involve autosomal recessive disorders, such that both parents are carriers of a pathogenic or disease-causing variant in the same gene. Carriers have no symptoms and in the face of a negative family history are unaware of their carrier status. Two carrier parents have a 25 percent chance of having a child affected with an autosomal recessive genetic disorder. Guidelines from the American College of Obstetricians and Gynecologists indicate that all women who are pregnant or planning a pregnancy have carrier screening for spinal muscular atrophy, hemoglobinopathies and cystic fibrosis.[2]

Fragile X syndrome testing is also included in expanded carrier screening. Fragile X syndrome is generally passed from mother to child. Carriers of Fragile X have an increased number of CGG repeats within the Fragile X gene. Carriers of a large number of CGG repeats may have mild symptoms such as learning problems. Some women with a large number of CGG repeats may have a higher chance of ovarian failure at a young age. Some men and women with an increased number of CGG repeats are at risk of developing an ataxia or movement disorder as they age. Fragile X syndrome is a complicated disorder.

The disorders included in the expanded carrier screen also have a high frequency in certain ethnic groups. Carrier screening for Tay Sachs disease has been available for decades.[3] The disease is very common among those of Ashkenazi Jewish ethnicity. About 1 in 27 Ashkenazi Jewish individuals are carriers for Tay Sachs, which means that without carrier screening, about 1 in 2900 children born would have this fatal incurable condition. The use of carrier screening for Tay Sachs has resulted in a 90 percent decrease in birth incidence for the Ashkenazi Jewish population.[4]

Several of the same disease-causing variants found in Ashkenazi Jewish carriers are also found in other ethnic groups, such as Cajuns, French-Canadians and Irish Americans, thus reinforcing

the need to extend carrier screening regardless of ethnicity. The same is true of the hemoglobinopathies. Sickle cell disease is one example of a hemoglobinopathy. 10 percent of the African American population has the sickle cell trait, but it is also common in those of Mediterranean, Middle Eastern and Indian ancestry as well as in those from the Caribbean and parts of Central and South America.

Clinical Indications

Ideally, carrier screening should be offered to women or couples preconception if possible, otherwise it should be offered early in pregnancy.[7] The expanded carrier screen is intended for those who are not considered at high risk for the targeted conditions. It may not be appropriate for those who have a personal or family history of one of the conditions or a partner who is a known carrier. Genetic counseling may be appropriate based on the patient's clinical or family history.

The expanded carrier screen is made up of two panels: a standard panel which targets disorders found in many ethnic groups (Table 1) and the additional disorders panel which targets disorders more frequently found in the Ashkenazi Jewish population (Table 2). The test order can be tailored to include the disorders most concerning for a woman or a couple. Couples who have had limited carrier screening in the past may opt to include those disorders for which they have not yet had testing.

Table 1. Standard Carrier Screen Panel

Bloom Syndrome
Canavan Disease
Cystic Fibrosis
Familial Dysautonomia
Familial Hyperinsulinism
Fanconi Anemia Group C
Fragile X Syndrome
Gaucher Disease
Glycogen Storage Disease Type 1a
Hemoglobinopathies
Maple Syrup Urine Disease 1b
Mucolipidosis IV
Niemann-Pick Types A and B
Sickle Cell Anemia
Spinal Muscular Atrophy
Tay-Sachs Disease
Thalassemia
Usher Syndrome IF
Usher Syndrome III

Table 2. Ashkenazi Jewish Disease Carrier Screen Panel

Bloom Syndrome
Canavan Disease
Familial Dysautonomia
Familial Hyperinsulinism
Fanconi Anemia Group C
Gaucher Disease
Glycogen Storage Disease Type 1a
Joubert Syndrome Type 2
Maple Syrup Urine Disease 1b
Mucolipidosis IV
Niemann-Pick Types A and B
Tay-Sachs Disease
Usher Syndrome IF
Usher Syndrome III

Table 3. Descriptions of Conditions Included in the Carrier Screen Panels

Condition Name	Description
Bloom Syndrome	Bloom syndrome is an autosomal recessive disorder characterized by short stature, skin rash with sun exposure and increased risk of cancer of any type. Affected individuals often have a high-pitched voice, distinctive facial features, learning disabilities, increased risk of diabetes and chronic obstructive pulmonary disease.
Canavan Disease	Canavan disease is an autosomal recessive disorder characterized by macrocephaly, lack of head control, hypotonia and developmental delays usually noted by a few months of age. The hypotonia becomes severe over time leading to failure to achieve independent sitting, ambulation, speech and feeding. Developmental delays are severe. Life expectancy for this severe form of Canavan disease is very poor. Mild/juvenile Canavan disease may be undiagnosed as it is characterized by mild developmental delay and does not affect life expectancy.
Cystic Fibrosis	Cystic fibrosis is an autosomal recessive multisystem disease that is characterized by chronic airway infection, pancreatic insufficiency, gastrointestinal dysfunction and male infertility. It is one of the most common single-gene disorders in the Caucasian population, with an incidence of about 1 in 3000. Symptoms typically present in early childhood, but, in a minority of cases, the diagnosis is not evident until adulthood. Life expectancy is shortened.
Familial Dysautonomia	Familial dysautonomia is an autosomal recessive disorder which affects the development and survival of sensory, sympathetic, and parasympathetic neurons. Affected individuals have gastrointestinal dysfunction, vomiting crises, recurrent pneumonia, scoliosis, altered sensitivity to pain, taste and temperature perception. Hypotonia contributes to delay in acquisition of motor milestones. Older individuals often have a broad-based and ataxic gait that deteriorates over time. Life expectancy is decreased.
Familial Hyperinsulinism	Familial hyperinsulinism is the most common cause of persistent hypoglycemia in infancy or early childhood. Symptoms including seizures, hypotonia, poor feeding and apnea may onset within hours or days of birth. In earlier onset, it causes serum glucose concentrations that are extremely low and can be difficult to manage. Early and aggressive intervention may be necessary to prevent brain damage from recurrent episodes of hypoglycemia. Childhood onset may occur in the first months or even years of life. Familial hyperinsulinism is generally an autosomal recessive disorder although it is important to note that there is a risk for offspring of a carrier

	father to develop congenital hyperinsulinism. There are also some individuals who are homozygous for one of the common variants (splice variant) and have no symptoms.
Fanconi Anemia Group C	Fanconi anemia group C is an autosomal recessive disorder that leads to early onset bone marrow failure. Characteristic clinical features include short stature, abnormal skin pigmentation, skeletal malformations of the upper and lower limbs, microcephaly, eye and genitourinary tract anomalies and congenital heart defect. Patients with FA have a high predispostion to cancer.
Fragile X Syndrome	Fragile X syndrome is the most common inherited cause of intellectual disability. It is an X-linked dominant condition characterized by moderate to severe mental retardation, macroorchidism and distinct facial features which include a long face, large ears and prominent jaw. It is caused by a trinucleotide (CGG) repeat expansion of greater than 200 repeats in the FMR1 gene. Carriers have more repeats than normal and are known as a premutation carrier. An intermediate,or "gray zone," allele has more than the normal range and less than the premutation range of repeats. Intermediate alleles do not cause symptoms and do not expand to full mutations in a single generation A carrier of larger premutations may have mild symptoms of Fragile X. Premutation carrier females are at increased risk for primary ovarian failure. Both premutation carrier females and males are at increased risk to develop Fragile X tremor/ataxia syndrome as they age (FXTAS). FXTAS is a late-onset, progressive development of intention tremor and ataxia often accompanied by progressive cognitive and behavioral difficulties including memory loss, anxiety, reclusive behavior, deficits of executive function and dementia. FMR1-related syndromes are very complex. Individuals considering carrier testing should be aware that they may discover risk factors about their own health beyond their carrier status.
Gaucher Disease	Gaucher disease is an autosomal recessive lysosomal storage disorder usually considered to have 3 main subtypes. Type I is the most common form of Gaucher disease and involves bone disease, hepatosplenomegaly, anemia and thrombocytopenia and lung disease but lacks primary central nervous system involvement. Of note, a significant portion of individuals with type 1 due to homozygous (two copies) of the common N370S variant are not diagnosed for years due to very mild or even absent symptoms. Such individuals may be detected via carrier screening. Types II and III have central nervous system involvement and neurologic manifestations. Type II has onset before age two years, limited psychomotor development and a rapidly progressive course with death by age two to four years. Type III may have early onset but has a slower progression, generally with a longer survival rate.
Glycogen Storage Disease Type 1a	Glycogen storage disease type 1a is characterized by accumulation of glycogen and fat in the liver and kidneys. Long-term complications of untreated GSDI include growth retardation resulting in short stature, osteoporosis, delayed puberty, gout, renal disease, pulmonary hypertension, hepatic adenomas with potential for malignant transformation, polycystic ovaries, pancreatitis and changes in brain function. Normal growth and puberty is expected in treated children.
Hemoglobinopathies	The hemoglobinopathies in this test include hemoglobin variants such as hemoglobin C, D and others, which may produce clinically significant anemia, spleen enlargement or sickling disease when paired with sickle cell trait (hemoglobin S) or a thalassemia variant. The hemoglobinopathies are generally autosomal recessive disorders. Hemoglobin evaluation of both reproductive partners is most effective for risk determination in high-risk groups.
Joubert Syndrome Type 2	Joubert syndrome type 2 is an autosomal recessive disorder. There are several forms of Joubert syndrome associated with multiple genes each with some variation in phenotype. This test is specific to Joubert syndrome type 2 due to variants in the TMEM216 gene. Joubert syndrome type 2 is characterized by a specific hindbrain malformation, which is referred to as the 'molar tooth sign' (MTS) on brain MRI, hypotonia, developmental delay, oculomotor apraxia, nephronophthisis (medullary cystic kidney disease) and breathing abnormalities.
Maple Syrup Urine Disease 1b	Maple syrup urine disease 1b is an autosomal recessive disorder, which presents with ketonuria, irritability and poor feeding a few days after birth and progresses

	quickly to deepening encephalopathy manifesting as lethargy, intermittent apnea and opisthotonus (hyperextension of the neck and spine). The urine of affected infants has a distinctive sweet odor. Without treatment via dietary restriction, this condition can be lethal.
Mucolipidosis IV	Mucolipidosis type IV is an autosomal recessive neurodegenerative lysosomal storage disorder. The most common form is severe and includes microcephaly, severe psychomotor delay with typically absent or limited speech, hypotonia with inability to walk, some with inability to sit, developmental delay, difficulty chewing and swallowing and progressive ophthalmologic abnormalities. Survival of those with the severe form may be into adulthood. There are a small percentage of affected individuals who are mildly affected, able to walk and may have mild vision problems.
Niemann-Pick Types A and B	Acid sphingomyelinase deficiency is comprised of the two types of Niemann-Pick disease. Both type A and B are autosomal recessive. Type A is a severe infantile form which involves hepatosplenomegaly, neurologic degeneration and interstitial lung disease resulting in death usually by 3 years of age. Type B is later-onset with milder manifestation with possible survival into adulthood. Intermediate cases also have been reported.
Sickle Cell Anemia	Sickle cell anemia is an autosomal recessive disease in which the red blood cells have an abnormal crescent shape, block small blood vessels and do not last as long as normal red blood cells. Symptoms include anemia and fatigue, episodes of pain including painful swelling of hands and feet, frequent infections and vision problems. Life expectancy is better than in the past but is still reduced.
Spinal Muscular Atrophy (SMA)	Spinal muscular atrophy is an autosomal recessive disease characterized by progressive muscle weakness, atrophy and paralysis. Additional common symptoms include poor weight gain with growth failure, abnormal swallow and speech, restrictive lung disease leading to respiratory failure, scoliosis, joint contractures and sleep difficulties. Onset and severity of symptoms is widely variable with onset ranging from before birth, in infancy (most common), or even in young adulthood although this is less common. There are clinical subtypes (I–IV) used historically which are primarily based on age at onset and disease severity but there is much overlap between these subtypes, even within the same family. There is also a wide range for life expectancy. In general, earlier onset involves more rapid progression and leads to shorter life expectancy.
Tay-Sachs Disease	Tay-Sachs disease is an autosomal recessive neurodegenerative disorder caused by hexosaminidase A deficiency. It is characterized by weakness, hypotonia, loss of motor skills, decreased attentiveness and increased startle response beginning between ages three and six months. Neurodegeneration progresses rapidly with seizures, blindness, spasticity and eventual total incapacitation and death, usually between 2 and 4 years.
Thalassemia (Alpha-Thalassemia or α-Thal and Beta-Thalassemia or β-Thal)	The thalassemias represent a wide spectrum of hematologic disorders that are characterized by a reduced synthesis of globin chains, resulting in microcytic anemia. Thalassemias are classified according to the globin chain affected, with the most common types being alpha-thalassemia (α-thal) and beta thalassemia (β-thal). Alpha-thalassemia is an autosomal recessive disorder caused by deletion of the α-globin genes. There are two clinically significant forms of α-thal, Hemoglobin Bart hydrops fetalis (Hb Bart) and Hemoglobin H (HbH). Hb Bart is the most severe form and is caused by deletion of all 4 α-globin genes. The severe anemia associated with Hb Bart leads to severe edema with congestive heart failure and can be detected prenatally. Very few infants survive this form of α-thal. HbH is the result of three α-globin gene deletions and leads to microcytic hypochromic hemolytic anemia (moderately severe anemia), hepatosplenomegaly, mild jaundice and sometimes bone changes such as overgrowth of the upper jaw and an unusually prominent forehead. HbH is generally milder than Hb Bart, but there have been severely affected infants with HbH as well. Individuals with one or two deletions of the α-globin genes are carriers for α-thal. Beta-thalassemia is an autosomal recessive disorder. There are two clinically significant forms: thalassemia major and thalassemia intermedia. Thalassemia major involves more severe anemia requiring regular blood transfusion. Bone

	marrow transplantation may be an option for treatment. Carriers of β-thal are also said to have thalassemia minor.
Usher Syndrome IF	Usher syndrome type IF is an autosomal recessive disorder characterized by congenital profound sensorineural hearing loss, progressive vision loss, poor balance leading to delayed motor milestones. Retinitis pigmentosa is the major cause of loss of vision. Cochlear implants enable hearing and speech development. The disease does not affect intelligence or lifespan.
Usher Syndrome III	Usher syndrome type III is an autosomal recessive disorder characterized by postlingual, progressive sensorineural hearing loss, late-onset RP and variable impairment of vestibular function (balance). It is very similar to Usher syndrome type 1F, but in general has a slower, later progression. Older patients may be candidates for a cochlear implant. The disease does not affect intelligence or lifespan.

Methodology

Carrier screening includes multiple tests to check for genetic mutations, but all the results are included in one report.

For Fragile X syndrome, the laboratory uses a polymerase chain reaction (PCR) analysis with restriction fragment length analysis.

For spinal muscular atrophy, the lab uses multiplex ligation-dependent probe amplification (MLPA).

For the hemoglobinopathies, which include alpha-thalassemia (α-thal), beta-thalassemia (β-thal), sickle cell anemia and other abnormal hemoglobins, the lab uses capillary electrophoresis with reflex to high-pressure liquid chromatography, as necessary.

For cystic fibrosis, Tay-Sachs disease, Bloom syndrome, Canavan disease, Niemann-Pick types A and B, mucolipidosis IV, Gaucher disease, Fanconi anemia group C, familial dysautonomia and the Ashkenazi Jewish disease carrier panel, the lab uses genotyping by mass spectrometry.

Test Advantages

The expanded carrier screen test is a comprehensive panel to identify individuals who may be at an increased risk of having a child with a genetic disorder. The main advantages of this test are that it targets the most common specific known disease-causing variants and summarizes the results in a single report. With rare exception, results will be unambiguous and clear-cut, with no variants of uncertain significance. The expanded carrier screen also offers health care providers and patients the option of customizing which disorders they want to include in their order for testing.

All of the genetic conditions included in the carrier screening are based on professional guidelines, and the test was developed in conjunction with Cleveland Clinic's Maternal-Fetal Medicine team.

By having a carrier screen test before or early in pregnancy, couples will have reproductive options available. The test can empower them with knowledge about potential inherited disorders.

Test Disadvantages

Fragile X syndrome testing may detect carriers of a premutation (increased number of CGG repeats) in the *FMR1* gene. Premutations in the *FMR1* gene may have future health implications for carriers. Female carriers of larger premutations may have mild symptoms of Fragile X. Premutation carrier females are at increased risk for primary ovarian failure. Both premutation carrier females and males are at increased risk to develop Fragile X tremor/ataxia syndrome as they age (FXTAS). FXTAS is a late-onset, progressive development of intention tremor and ataxia often accompanied by progressive cognitive and behavioral difficulties including memory loss, anxiety, reclusive behavior, deficits of executive function and dementia. FMR1-related syndromes are very complex. Individuals considering carrier testing should be aware that they may discover risk factors about their own health beyond their carrier status. Individuals with an intermediate allele will not be a risk for having children with Fragile X but will learn this information about their children's or grandchildren's reproductive risk.

Test Limitations

The carrier screen is not a diagnostic test. The analysis targets only the most common genetic variants that are known to cause inherited disorders. It does not analyze every variant for every gene, so it cannot eliminate carrier risk for every tested disorder. The carrier screen cannot guarantee that a child will not have one of these disorders due to a rare genetic variant inherited from each of the parents. It also does not cover every possible disorder that a child may inherit.

The expanded carrier screen may not be appropriate for those who have a personal or a family history of one of the conditions or a partner who is a known carrier. Genetic counseling may be indicated based on the patient's clinical or family history.

Limitations for Thalassemia

While there are many different mechanisms leading to thalassemia in populations from different areas of the world, this screening test will not detect molecular mechanisms. Risk for alpha-thalassemia trait is inferred from the mean cell volume and ferritin, not definitively detected. Alpha-thalassemia test results for individuals with single alpha gene deletions overlap those without deletions, reducing the rate of detection. Beta-thalassemia trait may not be detected in certain situations, such as undetected or untreated co-existent iron deficiency, co-inheritance of a delta chain variant and the presence of certain silent or mild beta thalassemia variants. Undetected thalassemia variants may cause significant disease in offspring if co-inherited with other hemoglobin variants from the other parent. Molecular testing of the alpha or beta globin genes are options to consider for further clarification of the reproductive risk, particularly if one

or both members of a couple are from a high-risk ethnic group. Hemoglobin evaluation of both reproductive partners is most effective for risk determination in the high-risk groups.

Limitations for Fragile X

This test cannot definitively determine the number of FMR1 alleles present, since two alleles of the same size appear as a single peak. Approximate FMR1 CGG repeat size accuracy is ±3 repeats. The test also does not provide accurate sizing in samples with >200 CGG repeats (full mutation) nor the methylation status of expanded alleles.

Limitations for Spinal Muscular Atrophy

Most spinal muscular atrophy (SMA) carriers have a single copy of SMN1 on one chromosome, while the other copy is deleted. This test cannot detect carrier status in individuals with two (or three) copies of SMN1 on one chromosome while the other chromosome has zero copies of SMN1 (2 + 0 genotype). At least 4% of the population has two SMN1 copies on one chromosome (higher in some ethnic groups). Small variants such as point mutations in SMN1 cannot be detected by this test. SMA sequence variants occur in approximately 5 percent of SMA patients and are not detected by this test. This test cannot detect other types of variants, including sequence variants, in the SMN2 gene. Use of this test to predict the likelihood of disease in offspring must also take into consideration that 2 percent of SMN1 disease-causing variants occur de novo rather than being inherited.

Limitation for Hemoglobinopathies

While many common hemoglobin variants may be definitively characterized by the test, others can be detected but may require additional testing for definitive characterization.

Test Results and Interpretation

Test results are either positive or negative. A positive result means the patient is a carrier for the genetic disorder(s). A negative result indicates that the screening did not find any of the variants included in the test, thus indicating a reduced carrier risk. The expanded carrier screen cannot eliminate the possibility that the patient is a carrier because the screening does not test for every possible variant, only the most common variants in each listed gene. The residual risk for each disorder will be summarized in the report for those disorders and ethnicities for which published information is available.

Health care providers will receive one PDF document that summarizes the results for all of the tested disorders.

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