

# GENETIC TESTING IN CLINICAL PRACTICE

## Goal:

Critically assess the availability of genetic testing for a particular condition, the quality of testing, the validity for a patient's circumstances, and implications of pursuing or declining such testing.

## After completing this activity participants will be able to:

- Identify applications of genetic testing in clinical practice
- Demonstrate knowledge of common genetic testing laboratory techniques
- Evaluate benefits, limitations, and risks of genetic tests
- Utilize clinical validity knowledge to select appropriate genetic testing
- Locate genetics professionals, services, and laboratories

## Professional Practice Gaps

In an effort to define what healthcare providers need to know about medical genetics, several organizations developed core competencies (NCHPEG, 2000; ASHG, 2001). However, because clinical genetics is a relatively young and evolving field of medicine, many practitioners received insufficient formal genetics education. As a result, they express a lack of confidence in their clinical genetics knowledge and a lack of confidence in their ability to provide genetic counseling.

## THE RELEVANCE OF GENETIC TESTING TO THE CLINICIAN

"The rate of increase of health care professionals trained and board-certified in medical genetics or genetic counseling has not kept pace with the rate of increase of genetic discovery and of potential demand for genetic tests. Other health care professionals will have to play a role or new models of testing will have to be devised if the demands are to be met" (Holtzman and Watson 1997).



- Genetic testing can be defined as a type of medical testing that identifies changes in chromosomes, genes, or proteins. The results of a genetic test can confirm or rule out a suspected genetic condition or help determine a person's chance of developing or passing on a genetic disorder (U.S. National Library of Medicine 2014).
- Genetic testing may provide information to support a diagnosis in an already symptomatic individual, to identify people at risk to have a child with a genetic condition, to indicate that an asymptomatic person is predisposed to develop a disorder later in life, or to direct management decisions based on predicted responses to therapeutics.
- As of October 2009, the GeneTests website identified genetic testing laboratories for 1,823 genetic diseases. Testing was available on a clinical basis for 1550 of these disorders, while 273 conditions had only research testing available.

- Genetic testing is expected to play an increasingly important role in medicine as testing becomes more realistic for a growing number of known inherited disorders and as the role of genetic factors in common disease development and response to drugs and other environmental exposures is elucidated.
- Knowledge of available tests, an understanding of how to best utilize them, and recognition of the potential impact genetic information can have on patients and their families will prove invaluable to healthcare providers.

## APPLICATIONS

### Common Uses for Genetic Testing

Some of the most common applications of genetic testing will be reviewed in the following pages:

- Prenatal screening
- Newborn screening
- Diagnostic testing
- Carrier testing
- Predictive testing
- Other: preimplantation genetic diagnosis, pharmacogenomics, identity testing (paternity, forensics, etc.), molecular oncology

## PRENATAL SCREENING

Nearly all pregnant women undergo some form of screening to determine if they have an increased risk for having a child with a congenital anomaly or genetic condition.

- A prenatal screen can be as simple as using a woman's age to estimate the fetal risk for Down syndrome or other chromosome abnormalities (specifically those involving a trisomy). It is generally accepted that women who are 35 years of age or older at delivery should be offered additional diagnostic testing (see below).
- Second trimester maternal serum screening for neural tube defects, Down syndrome, and trisomy 18 (a severe chromosome abnormality) is routinely offered to all pregnant women (ACOG 2007).
- First trimester screening options are also available, such as fetal nuchal translucency measurement and biochemical screening for Down syndrome and certain other birth defects.
- Fetal ultrasound may also be used to screen for physical anomalies in the fetus. These anomalies may be major birth defects (e.g., spina bifida, cardiac defect) that are isolated or part of a broader syndrome. Fetal ultrasound may also detect minor anomalies (e.g., choroid plexus cysts, hyperechoic bowel) that may simply be variations of normal development or subtle indications of a more major anomaly or syndrome.

Most screening tests simply indicate an increased risk, not a confirmed diagnosis. Thus, a pregnant woman who is determined to be at increased risk for having a fetus with an abnormality is typically offered additional diagnostic testing. While fetal ultrasound can be diagnostic of some physical anomalies, it is frequently necessary to obtain a fetal sample through such procedures as

amniocentesis, chorionic villus sampling (CVS), or periumbilical blood sampling (PUBS). These samples can be used for genetic studies, such as chromosome analysis, DNA testing, and biochemical studies. It is important to note, however, that many birth defects and genetic conditions will not be detected through routine screening and may not be diagnosable at all during pregnancy.

## NEWBORN SCREENING

### **Background**

All states have legislated newborn screening programs. Presently, however, there is no national screening program and limited guidance about how programs should be administered. Each of the 50 states (and the District of Columbia) is responsible for designing and administering its own newborn screening program based on available resources, interpretation of the appropriateness of disorder inclusion, and public advocacy (ACMG 2005). As a result, there is considerable variation in the conditions included in screening programs, the role of informed consent or dissent, and the logistics of screening, follow-up, diagnosis, management, and evaluation.

Until the advent of MS/MS, most states screened newborns for only a handful of conditions. Yet today, every state screens for or will soon be screening for at least thirty conditions, and some states screen for as many as fifty-seven. Many of these conditions were added to state screening panels in the past few years, in response to recommendations issued in 2005 by the American College of Medical Genetics (ACMG) (The President's Council on Bioethics December 2008).

You can view the conditions included in the National Newborn Screening Status Report maintained by the National Newborn Screening & Genetics Resource Center. Most states do not require parental informed consent for the screening process. The most recent Government Accountability Office report on Newborn Screening indicated that in 2003 thirty-three states have a provision allowing exemption from newborn screening for religious reasons, and 13 other states allow exemption for any reason.

## CURRENT NEWBORN SCREENING

### **Current Status**

The vast majority of the over 4 million infants born in the United States each year undergo newborn screening to detect specific disorders. The associated morbidity and/or mortality can be reduced if detected and treated early. Phenylketonuria was the first condition to be targeted for universal newborn screening in the 1960s and still serves as a good model for screening programs today.

There have been many different sets of guidelines for the establishment of newborn screening programs published over the years. Perhaps the most widely quoted are those published by Wilson and Jungner in 1968 as a companion report to the proceedings of the World Health Organization Scientific Group on Screening for Inborn Errors of Metabolism (see Table 1). These and other published principles essentially indicate that newborn screening should be performed for conditions of public health significance that have safe and effective treatments that provide a clear benefit with early detection. In addition, reliable and cost-effective screening and diagnostic testing should exist and an appropriate infrastructure should be present to manage the screening and diagnostic process.

### **Table 1: Principles of Early Disease Detection**

1. The condition sought should be an important health problem.
2. There should be an accepted treatment for patients with recognized disease.
3. Facilities for diagnosis and treatment should be available.
4. There should be a recognizable latent or early symptomatic state.
5. There should be a suitable test or examination.
6. The test or examination should be acceptable to the population.
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
8. There should be an agreed policy on whom to treat as patients.
9. The cost of case finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case-finding should be a continuing process and not a "once and for all" project.

(Friedman LF et al. 2013)

## **THE FUTURE OF NEWBORN SCREENING**

### **Future Directions**

The American Academy of Pediatrics convened a task force in 2000 to review the current state of newborn screening and make recommendations for the future (LLoyd-Puryear et al. 2006). One of the central concerns was the need for uniformity among states. As a result, the American College of Medical Genetics (ACMG) was commissioned by the Health Resources and Services Administration in part to make recommendations related to developing a uniform panel. That document is now available for public comment: Newborn Screening: Toward a Uniform Screening Panel and System.

Part of the screening debate stems from the relatively recent application of tandem mass spectrometry (MS/MS) to newborn screening. While newborn screening programs have historically included a very limited number of conditions (most commonly 8 or fewer), MS/MS measures many different metabolites, which allows screening for an extensive group of conditions simultaneously. Some of the conditions that can be routinely detected through MS/MS meet the criteria for inclusion in newborn screening programs, but others do not. It is possible to specifically exclude the detection of some metabolites, but it is not possible to avoid detecting some other conditions secondarily (ACMG 2005). Within the last five years, most screening laboratories in the United States have begun to use tandem mass spectrometry (MS/MS) as the principal tool for analyzing newborn blood samples (The President's Council on Bioethics December 2008). Refer to the ACMG/ASHG statement Tandem Mass Spectrometry in Newborn Screening for additional information.

## **DIAGNOSTIC TESTING**

### **Overview**

While genetic disorders have historically been diagnosed based on clinical findings, the ever-expanding and improving arsenal of genetic testing methodologies has made genetic testing the

preferred diagnostic tool in many clinical situations. Diagnostic testing is performed when a fetus, child, or adult has clinical findings suggestive of a genetic disorder. The purpose of testing is to confirm or rule out a diagnosis in a symptomatic individual -- similar to any other diagnostic laboratory study. Note that diagnostic testing is distinct from predictive genetic testing (discussed on the next page), which is done prior to symptom onset and is associated with more ethical, legal, and psychosocial concerns.

### **Is Genetic Testing the Best Option for Primary Diagnosis?**

The decision to use genetic testing for diagnostic purposes will depend on the availability, reliability, and cost of the various testing options based on the clinical circumstances. View the following examples to explore this question.

#### **Example 1**

The sweat chloride test is widely considered the gold standard for diagnosing cystic fibrosis (CF). For reliable results, this test must be performed in an experienced center with adherence to a strict protocol. Cystic fibrosis testing by DNA mutation analysis is also available, but there are over 1,000 different CF mutations. Testing for a panel of common mutations has variable sensitivity for disease-causing mutations based on ethnicity, but the sensitivity is generally considerably less than 100%.

- A child who presents with symptoms suggestive of CF should ideally first have sweat chloride testing in a reputable center, since the sensitivity is likely to be better than DNA mutation analysis.
- A fetus who presents with an echogenic area in the fetal abdomen that may be indicative of meconium ileus (a symptom of CF) is not a candidate for sweat chloride testing. Parents interested in prenatal diagnosis can instead be offered DNA mutation analysis on fetal cells obtained through amniocentesis, with appropriate counseling about the risks and limitations.

See Moskowitz et al. (2008) for more information about CF.

#### **Example 2**

Traditionally, the diagnosis of hereditary hemochromatosis has been made through some combination of clinical/biochemical evidence of iron overload, quantitative phlebotomy, and/or liver biopsy. More recently, molecular genetic testing for the 2 or 3 most common HFE mutations that can cause hemochromatosis has become widely available. Mutation analysis detects up to 90% of affected individuals in European populations. Individuals with a transferrin-iron saturation greater than 45% can now first be offered molecular genetic testing.

- If 2 disease-causing mutations are identified, the individual is at risk for developing complications associated with iron overload. Appropriate management can be undertaken without the added discomfort, risk, and cost of a liver biopsy.
- Individuals who have a single or no HFE mutation identified will likely require liver biopsy with histology and hepatic iron index for diagnosis.

See Bacon et al. (2011) for more information about hemochromatosis.

## **Should Genetic Testing Be Considered if a Clinical Diagnosis Has Already Been Made?**

Even when a diagnosis can be confidently made on clinical grounds, it may be useful to pursue genetic testing. Genetic test results can serve to confirm the clinical diagnosis and provide additional information that may be of use to the patient or family. View the following examples to explore this question.

### **Example 1**

Down syndrome can be reliably diagnosed by the experienced clinician based on dysmorphism and physical exam. The vast majority of children with Down syndrome have trisomy 21, a chromosome abnormality that does not confer a high risk of recurrence for other family members. About 3% to 5% of individuals with Down syndrome, however, have an extra copy of chromosome 21 due to translocation. This may represent a significant recurrence risk for family members. The underlying chromosome abnormality must be characterized for accurate genetic counseling.

### **Example 2**

Familial adenomatous polyposis is diagnosed clinically in an individual who has greater than 100 colorectal adenomatous polyps (as determined by pathology). While genetic testing to determine the underlying mutation may not alter the affected individual's management, a characterized familial mutation makes presymptomatic testing for at-risk family members much more reliable and inexpensive. Negative presymptomatic testing in at-risk family members allows the patient to avoid frequent invasive screenings, which begin at a young age.

# **CARRIER SCREENING**

## **Overview**

Carrier screening is designed to identify individuals who are at increased risk to have a child with a genetic disorder because the individual has a recessive gene mutation. Individuals who are carriers of recessive conditions are generally unaffected themselves because they have one normally functioning copy of a gene and one copy with a recessive gene mutation (genes typically come in pairs, with the exception of the sex chromosomes in males). Carriers are most commonly unaware that they are a carrier of a genetic condition.

Carrier screening may be done for autosomal recessive or X-linked recessive conditions.

- Autosomal recessive conditions: Both parents must carry at least one mutation (in the same gene) in order to have an affected child. (Rare exceptions occur such as in the case of de novo mutations.) Note that the mutations themselves do not have to be identical, they only have to occur in the same gene. For example, a deltaF508 mutation in the CFTR gene inherited from one parent and G542X mutation in the CFTR gene inherited from the other parent will result in cystic fibrosis in the child.
- X-linked recessive conditions: Only the mother needs to be a carrier of a gene mutation in order to have an affected male child. A male who has a single mutation is generally affected (not a carrier) because he has no second X chromosome. Thus, an affected male will have

only normal sons (who inherit their father's Y chromosome) and daughters who are carriers (who inherit their father's X chromosome with the mutation).

Carrier screening is offered to people who:

- Have a family history of a known or suspected recessive (autosomal or X-linked) condition
- Are of an ethnic background that is associated with an increased carrier risk

## THE TESTING PROCESS

Carrier screening may be done by DNA mutation analysis, biochemical analysis (particularly recessive disorders of metabolism such as Tay-Sachs disease), or other specialized testing such as hemoglobin electrophoresis. The sensitivity for genetic studies varies by methodology and ethnicity. While the sensitivity for biochemical and other gene-product studies is generally high, the sensitivity for DNA analysis rarely approaches 100%. Some of the most common carrier screening tests performed using DNA mutation panels are described in the following table. The typical indications for carrier screening are discussed below.

- **Family history:** When a family member is known to be a carrier of or affected with a recessive condition, it is always best to obtain documentation of the familial mutation(s) before testing the at-risk patient. A lab whose methodology is known to detect the familial mutation can then be selected and a positive or negative result can be clearly interpreted for the patient. If the familial mutation is unknown and carrier-screening results are negative, the lab will use the patient's a priori carrier risk (based on the family history) and the appropriate detection rate to calculate a revised risk.
- **Ethnicity:** When ethnicity-based carrier screening is performed, a positive result is generally clear. In cases where test sensitivity is reduced and the result is negative, the lab will often provide a revised carrier risk based on the patient's a priori risk to be a carrier and the detection rate as determined by ethnic background. Note that a patient's ethnicity should be provided to the lab doing carrier screening.

For additional information on ethnicity and autosomal recessive details for conditions that are more prevalent in specific ethnic groups, review this table.

Condition	Methodology	Ethnic Group	Detection Rate	Carrier Risk Before Testing	Carrier Risk With Negative Result
<b>Cystic fibrosis</b> <sup>1</sup>	Mutation panel: 25 recommended mutations for population-based screening	Ashkenazi Jewish	97%	1/29	~1/930
		European Caucasian	80%	1/29	~1/140
		African-American	69%	1/65	~1/207
		Hispanic-American	57%	1/46	~1/105

		Asian-American	Unknown	1/90	Unknown
<b>Tay-Sachs Disease</b> <sup>2</sup>	Mutation panel	Ashkenazi Jewish	94%	1/30	1/483
		Non-Jewish Caucasian	?	1/300	-
<b>Canavan disease</b> <sup>2</sup>	Mutation panel	Ashkenazi Jewish	98%	1/40	1/1950
		Non-Jewish Caucasian	40-50%	?	-

<sup>1</sup>ACMG, 2001 <sup>2</sup>ACOG, 2004

## PREDICTIVE TESTING

### Overview

Predictive genetic testing is used to identify asymptomatic individuals who are at increased risk to develop a particular genetic disorder at some point later in life. Most commonly, predictive genetic testing is undertaken because there is a known or suspected family history of an adult-onset condition.

It is useful to distinguish 2 different types of predictive genetic testing:

- **Presymptomatic testing:** An abnormal test result indicates that an individual will eventually develop symptoms of the disorder, assuming normal lifespan. Thus, the mutation that causes the condition has complete penetrance.
- **Predisposition testing:** An abnormal test result indicates that an individual has an **increased risk** for developing the condition but may never develop symptoms even if he or she has a normal lifespan. The predicted increase in risk can be highly variable (e.g., a 2-fold increased risk for Alzheimer disease, a 56% to 87% lifetime risk for breast cancer). Thus, the mutation that causes the condition has reduced penetrance.

(Holtzman and Watson, 1997)

## PREDICTIVE TESTING LIMITATIONS

### When Should Predictive Genetic Testing Be Used?

When used appropriately, predictive genetic testing can be a powerful tool for risk assessment and developing effective individualized management plans (Evans et al., 2001). However, the limitations of a predictive genetic test must be fully explored and understood by the clinician and the patient who is considering testing. These limitations may include the following:

- A negative result on predictive testing does not necessarily mean that an individual will never develop the condition. Example Hereditary nonpolyposis colon cancer (HNPCC) is caused by mutations in at least 4 different mismatch repair genes (MLH1, MSH2, MSH6, and PMS2). Sequence analysis of the 3 most commonly associated genes is available on a clinical basis

(MLH1, MSH2, and MSH6). The reported detection rates for HNPCC disease-causing mutations are difficult to accurately quantify because studies use different clinical criteria for diagnosis, test methodologies, and included genes. A clinically affected individual should always have testing first to determine if the HNPCC mutation is identifiable and to characterize the mutation for other family members' testing use. However, note that even an individual who tests negative for a known familial HNPCC mutation still has the general population risk for colorectal cancer and should follow population screening guidelines. See Kohlmann and Gruber (2004) for more information about HNPCC.

- An individual with an abnormal result may never develop symptoms if the identified mutation has reduced penetrance. Example Mutations in the APC gene may be associated with familial adenomatous polyposis (FAP), attenuated FAP (AFAP), or simply an increased risk for colorectal cancer. The vast majority of individuals who inherit a mutation known to be associated with classic FAP will develop numerous polyps and, eventually, colorectal cancer, if not treated. Those who inherit an APC gene mutation associated with AFAP typically develop fewer polyps and have a later average age at diagnosis for colorectal cancer, yet they still have a relatively high lifetime risk for colorectal cancer if untreated. Finally, individuals found to have a specific APC gene mutation, I1307K, simply have a 10% to 20% lifetime colorectal cancer risk. See Solomon and Burt (2004) for more information about FAP.
- Even when a mutation is known to have complete penetrance, often the age of symptom onset and/or severity cannot be predicted (variable expressivity). Example There is a single type of mutation that causes nearly all cases of Huntington disease. When an individual is found to have a Huntington disease mutation, the chance of developing the disorder is virtually 100% given a normal lifespan (complete penetrance). While onset typically occurs in the fourth or fifth decade of life and symptoms include progressive motor dysfunction, psychiatric disruption, and cognitive impairment, the age of onset and disease course of an individual can be highly variable even within a single family. See Haigh et al. (2005) for more information about Huntington disease.
- Results may be of limited clinical utility if effective and acceptable screening, surveillance, and/or treatments are not available. Example Clinically available genetic testing for hereditary breast and ovarian cancer (BRCA1/2) will detect up to 95% of disease-causing mutations. Women who inherit a BRCA1 or BRCA2 mutation have up to an 85% risk for being diagnosed with breast cancer and a 63% risk of ovarian cancer by 70 years of age. However, these studies were done in high-risk families and the actual risk for a woman with a BRCA1 or BRCA 2 mutation may be much lower. Management options for those with an identified mutation include heightened screening, chemoprevention, and/or prophylactic mastectomy and oophorectomy; however, the efficacy of these strategies has not been adequately studied in asymptomatic women with a BRCA1 or BRCA2 mutation. The efficacy of ovarian cancer screening is limited. Chemoprevention trials appear promising in some at-risk populations, but they are incomplete, there is conflicting data, and treatment is not without risk. Prophylactic mastectomy and oophorectomy appear to reduce risk in BRCA1 or BRCA2 mutation carriers, but this option is highly invasive and not widely acceptable. See Petrucelli et al. (2004) for more information about hereditary breast and ovarian cancer.

To date, predictive genetic testing has been used most commonly for conditions that are determined primarily by mutation(s) in a single gene. However, the majority of common conditions (e.g., cancer,

diabetes, cardiovascular disease) are caused by a complex interaction of genes and environment. As more is learned about these interactions, testing for identified predispositional genetic markers will become increasingly available. Individually, each of these markers may only modestly impact risk. The impact of various combinations of genetic markers and environmental factors may also not be fully understood. Some examples of such predispositional tests that are already clinically available are shown below with associated risk assessment information.

Predictive genetic testing is perhaps the most complicated and controversial application of genetic testing. In addition to the technical complexities, there are important ethical, legal, and psychosocial intricacies. For those genetic diseases that can be predicted presymptomatically but lack proven effective treatments, the decision about pursuing testing is often personal in nature instead of being based on medical necessity. For more information about the ethical and legal implications of predictive testing, consider taking our course on *Ethical and Legal Considerations in Genetic Testing*.

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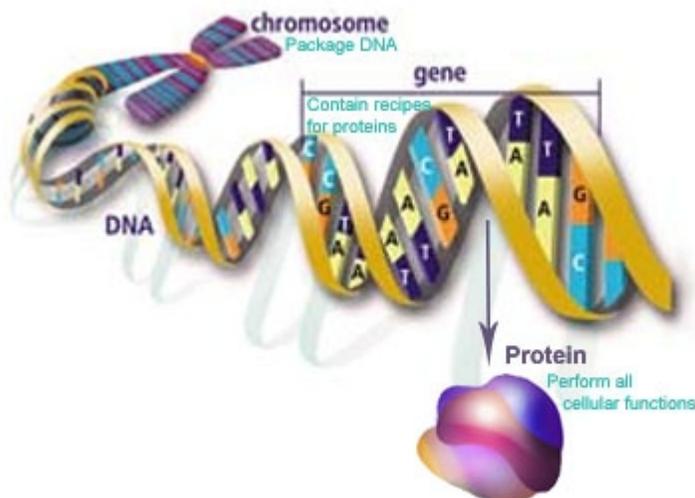
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## TECHNIQUES

### Genetic Testing Techniques - Introduction



(Image adapted from US Department of Energy, Human Genome Program, [http://web.ornl.gov/sci/techresources/Human\\_Genome/](http://web.ornl.gov/sci/techresources/Human_Genome/))

Genetic testing in the laboratory uses many approaches to gain information about a person's genetic makeup. Some tests evaluate chromosomes directly, others analyze DNA, and some assess the presence and/or function of specific gene products.

## CHROMOSOMES

### Chromosome Analysis

Chromosomes are present in most human cells and contain genes. With few exceptions, cells in the human body have 46 chromosomes that can be arranged into 23 pairs. One member of each pair is maternally derived and one member is paternally derived. Chromosome studies are most commonly used to confirm that the appropriate number of chromosomes is found in each cell and that individual chromosomes are normally formed (no missing, additional, or rearranged genetic information).

Chromosome analysis is a helpful tool when trying to determine the etiology of a patient's mental retardation, birth defects, or multiple miscarriages. Common chromosome studies include the following:

- Karyotyping
- Fluorescence in situ hybridization (FISH)

It is important to note that testing at the chromosome level offers neither information about the mutation status of individual genes located on the chromosomes nor information about gene products (proteins). For instance, it would not be appropriate to order "chromosome 7 testing for cystic fibrosis." Although the gene for cystic fibrosis is located on chromosome 7, evaluating the structure of chromosome 7 provides no information about DNA mutations in the cystic fibrosis gene. DNA or molecular analysis must be performed.

## MOLECULAR

### Molecular Analysis

The majority of genetic conditions are not caused by changes in chromosome number or structure. Instead, they are caused by a mutation in an individual gene that cannot be visualized during chromosome or FISH analysis. Just as chromosome analysis does not provide information about specific gene sequences/function, molecular analysis does not offer information about chromosome structure or number.

Molecular genetic (or DNA) testing can be designed to do the following: (Click on each for more information)

- Detect specific DNA mutations (mutation analysis).
  - This approach is most informative when there are a limited number of disease-causing mutations existing in the gene being studied. Results from a mutation panel will be reported as positive or negative for the mutation(s) studied.
- Test for any mutation that occurs in a defined region of DNA (mutation scanning, sequence analysis).

- This approach is most useful when mutations in the region of DNA being studied are very diverse, individually rare, and/or family specific. In such cases, it is not possible to develop mutation panels that are clinically useful for the majority of patients. This type of testing analyzes a much greater number of DNA base pairs than a mutation panel does. Therefore, it is labor intensive (particularly DNA sequencing) and, as a result, usually very expensive.
- Trace DNA markers through a family without directly identifying the disease-causing mutation (linkage analysis).
  - This approach traces markers that are physically close to the gene of interest in a family. Linkage analysis requires testing multiple family members and is costly and time consuming, but it may be the best option when other molecular approaches aren't available.

Molecular analysis only provides information about the gene(s) specifically analyzed. Therefore, when ordering a molecular genetic test for a patient, it is important that the correct gene be analyzed. For example, there are many different types of muscular dystrophy, and it would be useless to test for the common Duchenne muscular dystrophy mutations if the form of muscular dystrophy running in the family was limb-girdle muscular dystrophy. A result indicating that no Duchenne muscular dystrophy mutation was identified would provide no helpful information about the status of the gene responsible for symptoms in the family.

A tremendous number of different molecular techniques exist to evaluate DNA, and new, innovative processes are being developed regularly. It is not possible to discuss all of these techniques in this course. However, some of the techniques used very frequently are included below. The techniques listed here are often used in combination with one another to produce a clinically meaningful result.

Genetic testing for sickle cell anemia is a good example of the role of each of the following common techniques in the molecular testing process. Sickle cell anemia is the result of inheriting 2 copies of the gene mutation that causes hemoglobin S (HbS). HbS is most easily detected by hemoglobin electrophoresis when a blood sample is available. However, in a pregnancy at risk for HbS, amniocytes are more easily obtained than fetal blood. DNA testing for the HbS mutation can be done on amniocytes.

- Polymerase chain reaction (PCR)
- Restriction enzyme digestion
- Gel electrophoresis
- Southern blotting

## GENE PRODUCT

### Gene Product Analysis

Gene product analysis, like molecular genetic testing techniques, can provide information about gene function. Tests in this category analyze the product of a specific gene that may be the transcribed RNA or, most commonly, the translated protein. Protein-based testing either measures the quantity, quality, or function of the gene product present in a patient's sample. Gene product testing does not

provide any information about genes not specifically analyzed or about chromosome structure and number.

## TEST YOUR KNOWLEDGE

**What category of testing would be used to diagnose Down syndrome?**

Choose one

1. Chromosome analysis

- Feedback:

Correct. Chromosome analysis assesses entire chromosomes, and is useful in the diagnosis of Down syndrome.

2. Molecular analysis

- Feedback:

Incorrect. Try again. Molecular testing assesses single genes, not entire chromosomes, and is not useful in the diagnosis of Down syndrome.

3. Gene product analysis

- Feedback:

Incorrect. Try again. Gene-product analysis assesses protein quantity, quality, or function from individual genes, not entire chromosomes, and is not useful in the diagnosis of Down syndrome.

## TEST EVALUATION

### Genetic Test Considerations

Genetic testing has the potential to be a very powerful tool. However, it also has a great number of technical caveats and limitations. These unique qualities must be well understood to provide patients with best care.

When deciding whether genetic testing is warranted in a specific case, several factors should be considered.

### Clinical Availability of Testing

It should be noted that for many genetic disorders, no genetic test is available on a clinical basis. Diagnosis of these conditions relies on physical examination, family history, personal medical history, and other appropriate studies. Possible reasons for a lack of genetic testing for a given disorder include the following:

- One or more of the genes responsible for the condition have not been identified.
- Specific mutations in the relevant gene(s) have not been well characterized.
- The disorder is so uncommon or testing is of such little utility that there is limited interest in developing and offering testing on a clinical basis.

## Clinical Utility of Testing

The vast majority of genetic disorders lack a cure. Some disorders do have an effective treatment program; for the most part, however, clinicians are left managing symptoms. When there are no effective treatments, the use of genetic testing to diagnose a condition presymptomatically or to identify an individual as at risk to have an affected child is a complex and personal decision. Some patients may perceive testing as a benefit, even in the absence of treatment options. Benefits might include reduced uncertainty, avoidance of increased surveillance if a test is negative, and the possible avoidance of conception or birth of an affected child. These potential benefits must be weighed against such concerns as psychological harm and potential for discrimination (insurance, employment, and other forms). The genetic testing decision in these situations, therefore, is often based on a combination of personal values and motivations as well as medical management.

## Clinical Validity of Tests

Genetic test results can be challenging to interpret and require an understanding of the sensitivity, specificity, and positive predictive values of different methodologies. "Positive" test results do not necessarily mean that a person will develop symptoms of the condition tested for, and "negative" results may not rule out the possibility that the person is affected or at risk for developing a condition.

Listed below are the major factors impacting the clinical validity of a genetic test. The validity issues associated with molecular genetic testing are most pronounced and will be explored further in subsequent pages. However, it should be noted that each of these factors can also impact cytogenetic and gene-product testing. Regardless of the type of genetic testing ordered, the following factors should be considered when investigating all types of genetic testing:

- Sensitivity of the methodology
- Family member cooperation
- Penetrance of the disorder
- Variable expressivity
- Result interpretation for variants

These factors will be explored further in the coming pages.

## TEST YOUR KNOWLEDGE

Which of the following is **TRUE** regarding genetic test results?

Choose one

1. A positive result typically can be used to predict disease course and severity.

- Feedback:

Incorrect. Try again. In order for a genetic test result to predict disease course and severity, there must be complete penetrance of the mutation, no variable expressivity, and/or complete information about genotype-phenotype correlations. This is currently uncommon in genetic testing situations.

2. Counseling and informed consent should be provided before and after testing.

- Feedback:

Correct. Because genetic testing has many caveats, it is typically best to discuss the risks, benefits, and limitations of testing in a person prior to ordering testing. The impact that a positive or negative result has on risk interpretation and management options should then be thoroughly explained after the results are known.

3. A negative result eliminates disease risk.

- Feedback:

Incorrect. Try again. In order for a negative genetic test result to eliminate disease risk, there must be no other cause for the disease (other genes untested or unknown, environmental factors, etc.), and the test must have a sensitivity of 100% for disease-causing mutations. This is relatively uncommon in genetic testing situations.

4. Results are always conclusive.

- Feedback:

Incorrect. Try again. There are unfortunately many reasons why genetic tests may be inconclusive. These reasons include mutations of unknown significance, gene product levels that fall in indeterminate ranges, unknown or incomplete information about mutation penetrance, or a negative result in an unaffected person where no familial mutation has been characterized, etc.

## SENSITIVITY

### **A negative genetic test result may not rule out the presence of a mutation**

Factors Influencing Test Sensitivity Include the Following:

- Locus heterogeneity
- Allelic heterogeneity
- Methodology (mutation analysis, sequence analysis, etc.)
- Ethnicity

The sensitivity of DNA testing is often reduced because a specific test will only be able to identify certain mutations. This reduced sensitivity is particularly common for mutation analysis, although even sequence analysis may not be able to detect some mutations (large deletions or genomic rearrangements). For conditions with many different possible mutations, achieving a sensitivity of 100% may not be possible because of the expense and time that would be required. Physicians, patients, and laboratories performing the analyses must choose from available methodologies, balancing both sensitivity and cost. For tests with reduced detection rates, a Bayes statistical analysis can often be performed. Bayesian analysis takes into account a patient's risk prior to testing as well as the detection rate of the test and generates the remaining risk of either being affected with (or a carrier of) the disorder tested for, based on a negative result. It is important that patients be informed of the sensitivity of any test being considered so that a negative result is not falsely reassuring.

For more information about how to determine an individual's residual carrier risk (after a negative test result) when the test sensitivity is reduced, review our brief Bayes analysis discussion in the 'Related Resources' section.

# CLINICAL EXAMPLES

**Clinical Example of Decreased Sensitivity:** Cystic fibrosis (CF) is an autosomal recessive disorder characterized most commonly by chronic pulmonary disease and pancreatic insufficiency with onset in early childhood. Over 900 mutations in the CF gene have been identified, but most are quite rare. A consensus statement published by several national organizations recommended that laboratories test for a pan-ethnic panel consisting of mutations occurring with a frequency of at least 0.1% in the US population. The original panel contained 25 mutations. This Working Group has since omitted two mutations from this panel in light of more recent scientific literature on the frequency of these two mutations. Using this mutation panel, the detection rate for disease-causing CF mutations varies widely between different ethnic groups. Individuals interested in learning if they are unaffected carriers of CF must be aware that genetic testing can reduce carrier risk but never to 0%. See the table below for reduction in risk to be a carrier for CF after testing negative.

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## Estimated Carrier Risk

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Ethnic Group	Detection Rate	Before Test	After Negative Test
Ashkenazi Jewish	97%	1/29	~1 in 930
European Caucasian	80%	1/29	~1 in 140
African-American	69%	1/65	~1 in 207
Hispanic-American	57%	1 /46	~1 in 105
Asian-American	Unknown	1/90	Unknown

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Note: Because the detection rate is not 100% for any ethnicity, diagnosis of the condition should not be based solely on DNA testing when evaluating children suspected of having CF. Sweat chloride analysis is considered to be a better diagnostic tool.

(Adapted from Grody et al. 2001)

Examples of Genetic Disorders With Reduced Sensitivity Molecular Tests (Common Detection Rate):

- Cystic fibrosis (varies based on ethnicity)
- Duchenne/Becker muscular dystrophy (70%)
- Hemophilia A (30%)
- Hereditary hemochromatosis (90% Caucasians)

**Examples of Genetic Disorders With Molecular Test Sensitivities Approaching 100%:**

- Achondroplasia
- Alpha-1-antitrypsin deficiency
- Fragile X
- Huntington disease
- Myotonic dystrophy
- Sickle cell anemia

# FAMILY MEMBER COOPERATION

## **Some tests with reduced sensitivity require family member cooperation for optimal result interpretation.**

Even if a molecular genetic test does not have a 100% detection rate, it may still be possible to rule out the presence of a mutation with family member cooperation. If a clinically diagnosed family member undergoes genetic testing and the underlying mutation(s) is identified, other family members can then be tested for that specific gene mutation(s). If biological relatives are negative for the specific mutation known to cause the disorder in the family, the results are very reliable and effectively rule out the condition (or carrier status). Conversely, if a mutation is not identified in an affected family member, testing by that methodology will be of no use to other (affected or unaffected) family members. Testing an affected family member first, whenever possible, is critical to the optimal use of genetic testing.

**Clinical Example:** Autosomal dominant polycystic kidney disease (ADPKD) is an adult-onset genetic condition characterized by bilateral polycystic kidneys, cysts in other organs, and end-stage renal disease by 60 years of age in about half of affected individuals. The responsible mutation can be identified in about 50% to 75% of cases. If an unaffected person with a family history of ADPKD tests negative for a mutation, it is impossible to know if that person is truly negative for ADPKD or if the familial mutation is simply one of the 25% to 50% that cannot be detected through current technologies. However, if a clinically affected family member is tested first and the disease-causing mutation characterized, other family members can be tested for that specific familial mutation. If other family members are negative, ADPKD can be reliably ruled out.

## **The cooperation of multiple family members is required for linkage analysis.**

Linkage analysis essentially traces DNA markers associated with the presence of disease in multiple family members. The marker pattern can then be used to predict mutation status of individuals, without directly identifying the disease-causing mutation. Family member availability and cooperation is paramount to successful linkage analysis. Potential barriers include the following:

- Key family members unwilling to participate
- Small families
- Families with few or no living, affected individuals

In general, the more family members available for testing, the greater the likelihood of an informative result. Because linkage analysis results are based on markers in proximity to the disease-causing mutation and not the actual mutation, results are generally expressed in probabilities.

# PENETRANCE

## **Presence of a mutation may not mean disease.**

In some circumstances, a genetic test may indicate the presence of a disease-causing mutation, but the person does not develop the disorder. This is a result of ***incomplete penetrance***, which simply means that a gene mutation does not result in clinical symptoms in 100% of individuals who possess the mutation.

**Clinical Example:** Hereditary hemochromatosis is an autosomal recessive disorder of iron metabolism characterized by increased iron absorption and subsequent accumulation in body tissues. Presenting symptoms vary widely but most commonly include fatigue, abdominal pain, weakness, and joint pain. If untreated, symptoms generally develop between 40 and 60 years of age; late findings include hepatic cirrhosis, increased skin pigmentation, diabetes mellitus, cardiomyopathy, and arthritis. Treatment is therapeutic phlebotomy and has a good prognosis if therapy is initiated prior to tissue damage. Most clinically affected individuals are either a homozygote for the C282Y mutation (~80%) or are compound heterozygotes for C282Y and another mutation (H63D or S65C) in the HFE gene (Mura et al., 1999). The differential diagnosis of hereditary hemochromatosis has traditionally been considered for a patient only after the onset of symptoms of iron overload and/or biochemical evidence (serum transferrin-iron saturation and serum ferritin concentration). In a symptomatic individual with elevated serum transferrin-iron saturation, molecular genetic testing for common HFE mutations can support the diagnosis without the need for liver biopsy.

Interpretation of molecular studies is much less clear-cut when a patient does not exhibit classic clinical findings or when testing is ordered in the absence of any symptoms (such as when done due to family history). This is because HFE mutations exhibit reduced penetrance. The C282Y mutation appears to be the most penetrant of 3 common mutations associated with disease (C282Y, H63D, and S65C). However, even homozygotes for the mutation are not guaranteed to develop symptoms. Olynyk et al. (1999) found that of 3,000 Australian patients homozygous for C282Y, all had elevated transferrin-iron saturation, although only half had elevated serum ferritin on 2 occasions in 4 years. Olynyk's observations suggest that not all homozygotes for C282Y develop iron overload. Compound heterozygotes for C282Y and the much less penetrant mutation H63D (C282Y/H63D) appear to have only a 0.5% to 2% chance for developing clinical symptoms of iron overload (Kowdley et al., 2004). Homozygotes for the H63D mutation (H63D/H63D) are expected to have even lower likelihood of disease.

**Examples of Genetic Conditions That Exhibit Incomplete Penetrance:**

- Factor V Leiden heterozygotes
- Fragile X in females
- Hereditary breast and ovarian cancer
- Hereditary hemochromatosis
- Hereditary nonpolyposis colorectal cancer

**Examples of Genetic Conditions Approaching 100% Penetrance:**

- Achondroplasia
- Duchenne muscular dystrophy
- Familial adenomatous polyposis
- Fragile X in males
- Huntington disease
- Sickle cell anemia

## VARIABLE EXPRESSIVITY

**A positive result may have limited prognostic ability.**

In some circumstances, genetic tests have a high sensitivity and penetrance, yet the results will have limited prognostic capability. In this situation, result interpretation is impacted by variable expressivity.

Variable expressivity is common to many diseases, meaning that there is much variation in the clinical course of disease, even within the same family. Knowledge gained during genetic testing of a specific genotype, therefore, may not be predictive of the course of disease (phenotype) for any one patient (genotype-phenotype correlation).

**Clinical Example:** Neurofibromatosis (NF) type 1 is an autosomal dominant disorder that is diagnosed when clinical criteria established by the NIH are met. At least two of the following are required for diagnosis:

- 6 or more cafe au lait spots over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals
- 2 or more neurofibromas of any type, or 1 plexiform neurofibroma
- Freckling in the axillary or inguinal regions
- Optic glioma
- 2 or more Lisch nodules (iris hamartomas)
- A distinctive osseous lesion such as sphenoid dysplasia or thinning of long bone cortex with or without pseudarthrosis
- A first-degree relative (parent, sibling, or child) with NF1 as defined by the above criteria

(Ferner et al. 2007)

Molecular testing is not commonly performed for patients with neurofibromatosis because the diagnosis is usually made from clinical features. However, there are occasions when molecular testing may be useful. For example, a parent with NF may wish to know if his or her child will inherit the condition, and the parent may elect to pursue prenatal testing. Once a mutation has been characterized in the parent with NF, that mutation can be tested for in the fetal sample (obtained either through chorionic villus sampling or amniocentesis). Almost all people with a neurofibromatosis mutation will develop some clinical symptoms, but presentation is extremely variable. Most will develop cafe au lait spots and unusual freckling patterns. Some individuals also develop learning disabilities, life-threatening tumors, or other serious complications. Parents must be made aware that their personal experience with neurofibromatosis does not predict their child's experience.

## VARIANTS

### **Genetic Variants With Unknown Clinical Impact**

**An abnormal result may not be fully interpretable.**

Some genetic tests identify DNA sequence variants that have not been previously characterized; therefore, interpretation of the results is difficult. The occurrence of DNA sequence variation is explained, in part, by normal genetic diversity. That is, people do not have the exact same genetic constitution (unless they are identical twins). Some genetic changes allow for individual differences and do not cause disease. A benign genetic variant that occurs in more than 1% of the population is called a polymorphism. The human genome has not been studied fully enough for each polymorphism to have been characterized. Therefore, a patient may have an identified genetic

change in a disease-causing gene, but it is possible that the laboratory will not know if that mutation is disease-causing or a simple benign polymorphism.

**Clinical Example:** BRCA1 and BRCA2 gene sequencing is the most commonly used test for individuals at risk for hereditary breast and ovarian cancer. Sequencing technology has the ability to evaluate each nucleotide in a gene, base by base. Because the methodology evaluates each nucleotide, it also bears the risk of identifying sequence changes that have not been previously described. It is difficult to confidently predict the clinical impact of these variants. The only commercial laboratory offering sequencing for BRCA1 and 2, Myriad Genetics, reports results as follows:

<b>Result</b>	<b>Interpretation</b>	<b>Clinical Significance</b>
<b>Positive for a deleterious mutation</b>	Predicted to be clinically significant	Patient at increased risk for breast/ovarian and associated cancers
<b>Genetic variant, suspected deleterious</b>	Suspected to be clinically significant	Patient thought to be at increased risk for breast/ovarian and associated cancers
<b>Genetic variant, favor polymorphism</b>	Not suspected to be clinically significant	Patient not thought to be at increased risk for breast/ovarian and associated cancers
<b>Genetic variant of uncertain significance</b>	Not known if clinically significant	Not known if patient is at increased risk for breast/ovarian and associated cancers
<b>No deleterious mutation detected</b>	Patient negative for genetic changes screened for	Result does not eliminate possibility that patient has an uncommon gene change in BRCA1 or BRCA2 <b>or</b> a mutation in another cancer-causing gene
<b>Specific variant or mutation not identified</b>	Patient negative for genetic changes found in a previous family member	Clinical impact dependent upon family history

## BEFORE YOU ORDER

### Ordering Genetic Tests

When ordering a test that is unfamiliar, it is always wise to discuss the indications, limitations, and testing process directly with the laboratory performing the analysis prior to obtaining a patient sample.

## **Locating a Laboratory**

Testing for common genetic disorders, such as cystic fibrosis or hereditary hemochromatosis, is often done in large commercial laboratories. However, for the majority of genetic disorders, testing is done in smaller, specialized laboratories. To identify laboratories that perform genetic testing, both on a clinical and a research basis, a resource commonly used is the GeneTests website. GeneTests may be searched by disease name, gene name, and laboratory name. In addition, GeneTests also provides many other valuable resources including the following:

- A database of genetic clinics searchable by state and specialty
- Thorough reviews of many different genetic disorders (GeneReviews)
- Other educational materials designed for healthcare providers and the public about genetic counseling and testing.

The site is publicly funded, and there is no cost to use it.

## **Testing Process**

In addition to the many serious clinical validity issues to consider with genetic testing, there may also be significant practical issues that should be identified prior to initiating testing. For instance:

- Genetic testing samples often require specific collection and handling. If not obtained and handled correctly, precious samples can be rendered useless.
- Almost all genetic tests are significantly more expensive than routine laboratory analyses. Insurance coverage cannot be assumed.
- Genetic tests typically take longer than standard laboratory analyses because of the methodologies used. The time required often cannot be significantly altered. For situations with time constraints (such as in the case of prenatal diagnosis), appropriate planning is required to ensure ample time for testing.
- Supporting information (ethnicity, family history, medical records, other test results) or samples from other family members may be required to interpret the results.
- Not all laboratories that offer genetic testing are of equal quality and should be carefully assessed.

For a more thorough review of points to consider when arranging genetic testing, please refer to the GeneTests Web page

# **GENETIC COUNSELING**

The patient should be provided genetic counseling if the following criteria have been met:

- A patient is determined to be an appropriate candidate for genetic testing
- The availability of testing confirmed
- The benefits and limitations of different methodologies investigated

Ideally, patients should receive:

- Pre-test counseling
- Informed consent
- Post-test counseling

The goal of pre-test genetic counseling is to effectively convey all information that a patient needs to know in order to make an informed decision (**informed consent**) about pursuing or declining genetic testing. Patients should be encouraged to make decisions consistent with their individual and familial values and beliefs. Depending on the test and the results, patients may also benefit from post-test counseling. For an in-depth discussion of both the genetic counseling goals and process, consider taking the *Introduction to Genetic Counseling* course on this site.

In complicated situations or for busy physicians, it may prove too time consuming to personally provide genetic counseling to patients. Physicians may instead prefer to refer patients to a local genetics professional.

To easily locate local genetics professionals, organizations provided in 'Related Resources' maintain searchable databases of genetic counselors, physician geneticists, and other genetics professionals.

## INFORMED CONSENT

In Order for the Patient to Give Fully Informed Consent, All of the Following Should Be Discussed and Understood:

- Natural history of the condition for which testing is being considered
- Inheritance pattern of the condition and the patient's specific risk
- All evaluation (genetic testing, other clinical and laboratory testing) and healthcare management options
- Potential medical and personal ramifications of pursuing or declining testing
- Benefits, risks and limitations (sensitivity, specificity, penetrance, etc.) of the genetic test(s) and/or procedures to obtain samples
- If applicable, reproductive choices
- If applicable, patient-oriented support group information

## CASE EXAMPLE

### Clinical Scenario

You will now have an opportunity to apply your knowledge about genetic testing to the selection of the appropriate test for the patient described below. This scenario is representative of a clinical situation in which genetic testing is commonly misordered. It requires an understanding of the benefits and limitations of 3 different genetic testing methodology options.

**Case Introduction:** Rebecca is seeing you as a new obstetrics patient today. This is her first pregnancy, and she is currently about 6 weeks' gestation. In reviewing her screening form, you learn that she is a 28-year-old, healthy, Ashkenazi Jewish woman with no family history of congenital anomalies or known inherited disorders. Based on ACOG recommendations, you realize that she should minimally be offered carrier screening for Tay-Sachs disease, Canavan disease, familial dysautonomia and cystic fibrosis, although there are additional carrier tests for conditions common in the Ashkenazi Jewish population that should be considered. When investigating the tests available at the laboratory you use most commonly, you learn that there are 3 different carrier-screening tests for Tay-Sachs

(hexosaminidase A deficiency) from which to choose. The laboratory provides you with the following information about each of the tests.

**Hexosaminidase A, Serum**

**Methodolgy Characteristics**

Detection rate >99%  
 May not detect rare variants (B-I variant or the activator protein deficiency)  
 False positives possible due to pseudodeficiency mutation

**Required Documentation**

Tay-Sachs questionnaire (family history, ethnicity, pregnancy status, reason for testing)  
 Signed informed consent

**Sample Requirements**

1-3 ml serum frozen within 1h of collection

**Resulting**

Noncarrier ≥ 55%  
 Inconclusive 51-54%  
 Carrier 35-50%  
 ~3% of Jewish and 32% of non-Jewish enzyme-defined carriers have a pseudodeficiency mutation that causes false positive enzyme tests but does not increase risk for affected offspring

**Special Considerations**

Not valid in pregnant women or those taking oral contraceptives

**Cost**

\$110

**Hexosaminidase A, Leukocytes**

**Methodolgy Characteristics**

Detection rate >99%  
 May not detect rare variants (B-I variant or the activator protein deficiency)  
 False positives possible due to pseudodeficiency mutation

**Required Documentation**

Tay-Sachs questionnaire (family history, ethnicity, pregnancy status, reason for testing)  
 Signed informed consent

**Sample Requirements**

7 ml whole blood in yellow top (ACD solution A or B) tube refrigerated or transported with cool pack, received in the lab within 48h of collection

**Resulting**

Noncarrier ≥ 58%  
 Inconclusive 53-57%  
 Carrier 35-52%  
 ~3% of Jewish and 32% of non-Jewish enzyme-defined carriers have a pseudodeficiency mutation that causes false positive enzyme tests but does not increase risk for affected offspring

**Special Considerations**

None.

**Cost**

\$225

**Tay-Sachs, DNA mutation analysis**

**Methodolgy Characteristics**

Mutation Panel: 1278insTATC, 1421+1G-C, G269S, R247W and R249W  
 Detection rate: ~95% Ashkenazi Jewish individuals, ~20% for disease-causing mutations in non-Jewish Caucasians

**Required Documentation**

Tay-Sachs questionnaire (family history, ethnicity, pregnancy status, reason for testing)  
 Signed informed consent

**Sample Requirements**

3-7 ml whole blood in lavender top (EDTA) tube, room temperature

**Resulting**

If positive for mutation, different reports for infantile-onset disease mutations (1278insTATC, 1421+1G-C), adult-onset mutation (G269S) and pseudodeficiency mutations (R247W, R249W)

**Special Considerations**

None

**Cost**

\$285

# TESTING DECISION

## Which Tay-Sachs test would you choose for Rebecca?

Choose one

1. Hexosaminidase A, serum

- Feedback:

This is NOT the best choice. Although this test has a very high detection rate, it is not valid in pregnant women. The best choice is Hexosaminidase A, leukocytes. This test is felt by many to be the best initial Tay-Sachs carrier screen for pregnant women because the detection rate is high and the cost is relatively low. No further testing is required for individuals who are negative. However, for those who are found by this methodology to be in the carrier range, follow-up with DNA analysis is recommended to rule out a pseudodeficiency mutation and to characterize the mutation for those who may wish to pursue prenatal diagnosis. Potential drawbacks of hexosaminidase A, leukocyte testing are the specimen handling requirements and the possibility of an inconclusive result, requiring additional testing to resolve. In situations where there are serious time constraints, consider doing enzyme and mutation testing concurrently.

2. Hexosaminidase A, leukocytes

- Feedback:

This is the best choice. This test is felt by many to be the best initial Tay-Sachs carrier screen because the detection rate is high and the cost is relatively low. No further testing is required for individuals who are negative. However, for those who are found by this methodology to be in the carrier range, follow-up with DNA analysis is recommended to rule out a pseudodeficiency mutation and to characterize the mutation for those who may wish to pursue prenatal diagnosis. Potential drawbacks of hexosaminidase A, leukocyte testing are the specimen handling requirements and the possibility of an inconclusive result, requiring additional testing to resolve. In situations where there are serious time constraints, consider doing enzyme and mutation testing concurrently.

3. Tay-Sachs, DNA mutation panel

- Feedback:

This is NOT the best choice. However, it is a reasonable choice. The detection rate for Rebecca is about 95%, given that she is Ashkenazi Jewish. If she were of a different ethnic background, mutation analysis would not be a good testing choice. The benefits of DNA testing are that a positive result requires no further action and specimen handling is simple. The drawbacks are that the cost is higher and the detection rate is somewhat lower than enzyme testing. If Rebecca had a high risk -- based on family history -- for being a carrier and was negative by DNA, consider following up with enzyme testing. In situations where there are serious time constraints, consider doing enzyme and mutation testing concurrently.

# TEST RESULTS

Rebecca is found to be a carrier by hexosaminidase A, leukocytes (hexA activity 48%). She also had the recommended DNA testing in follow-up to rule out a pseudodeficiency mutation. DNA testing identified a single copy of the 1278insTATC mutation, the most common Tay-Sachs mutation in the Ashkenazi Jewish population, consistent with being an unaffected carrier. Rebecca's husband, John, is of English and German descent, with no known Ashkenazi Jewish ancestry. Understanding that his chance to be a carrier of Tay-Sachs is low given his ethnic background, the couple would still like to have John carrier-screened for reassurance.

Choose one

1. Hexosaminidase A, serum

- Feedback:

This is the best choice. This test has a very high detection rate regardless of ethnicity, the cost is low, and pregnancy is not an issue in John's case. If John had an abnormal hexosaminidase A result, DNA testing would be very important in follow-up to rule out a pseudodeficiency mutation (32% of non-Jewish, enzyme-defined carriers).

2. Hexosaminidase A, leukocytes

- Feedback:

This is NOT the best choice. However, it is a reasonable choice. Hexosaminidase A, leukocyte testing has a high detection rate, equal to serum. It is, however, more expensive, with somewhat more complicated specimen handling in comparison, and the benefit of being valid in pregnant women or those using oral contraceptives is not required for John.

3. Tay-Sachs, DNA mutation panel

- Feedback:

This is NOT the best choice. Performing Tay-Sachs DNA mutation analysis as an initial carrier-screening method is not recommended for people who are not Ashkenazi Jewish, as the detection rate is poor (about 20% for disease-causing mutations). Given that one member of the couple has already been determined to be a carrier, the detection rate for the other should be as high as possible. The best choice for John would be hexosaminidase A, serum. This test has a very high detection rate regardless of ethnicity, the cost is low, and pregnancy is not a concern. If John had an abnormal hexosaminidase A result, DNA testing would be very important in follow-up to rule out a pseudodeficiency mutation (43% of non-Jewish, enzyme-defined carriers).

## SUMMARY

- Clinical genetic testing is currently available for over 1550 genetic conditions ([Genetests.org](http://Genetests.org)). In the future, it is likely that a greater number of tests will be available and that those tests will have a wider range of application.
- Genetic tests are commonly used for prenatal screening and diagnosis, newborn screening, diagnostic testing, carrier testing, and predictive testing.

- Genetic tests can be divided into 3 broad categories: chromosome analysis, molecular testing, and protein product testing.
- There are many important limitations of genetic testing in terms of availability, clinical utility of results, and methodology considerations, which must be understood in order to responsibly provide genetic testing. Result interpretation can be difficult, requiring integration of the technical limitations, family history, and clinical presentation.
- Genetic testing, even for a routine indication, has the potential for significant ethical, legal, and psychosocial implications for the patient that must be considered in the test decision-making process.

## RESOURCES AVAILABLE THROUGH THIS MODULE:

- [A Brief Primer on Genetic Testing](#)  
This webpage provides basic information on genetic testing including what genetic testing is, what tests are available, and what the future holds for these tests.
- [American College of Medical Genetics: Membership Directory](#)  
The American College of Medical Genetics is composed primarily of doctoral (MD, PhD, DO) and master's level (genetic counselors) medical genetics professionals. This is a searchable database of all ACMG members by name or location.
- [Calculating Revised Carrier Risks -- Bayes Analysis](#)   
Bayesian analysis allows one to compare the likelihood of 2 outcomes based on recognized facts. This approach can be used to calculate a variety of probabilities important in medical genetics, including the likelihood of being a carrier following a negative carrier screen. To perform a Bayes analysis in this situation, one must first define the individual's initial carrier risk (prior probability) -- usually based on pedigree calculations or ethnicity data. That initial risk can then be modified based on the negative carrier screen result to determine a revised carrier risk (posterior probability). An example of a common calculation is shown below.
- [Enzyme Analysis](#)   
Gene products may act as enzymes in the body. Measurement of enzyme activity in the laboratory can provide information about the functionality of the gene producing the enzyme.
- [Ethnicity and Autosomal Recessive Conditions](#)   
Some autosomal recessive disorders are known to occur more frequently in people of specific ethnic backgrounds.
- [Examples of Available Genetic Tests](#)   
As of October 2009, the GeneTests website genetic testing database listed 1,550 genetic disorders with clinically available genetic tests.
- [Fluorescent in situ hybridization \(FISH\)](#)   
Fluorescent in situ hybridization (FISH) technology is useful for the detection of structural chromosome abnormalities too small to be noted on a routine karyotype.
- [Gel Electrophoresis](#)   
Gel electrophoresis is the process of moving substances (e.g., DNA, RNA, or protein) through a gel using an electric current. Smaller pieces move through the gel more quickly than larger ones. The different rate of travel through the gel permits separation of different-sized

substances. Molecular testing most commonly uses gel electrophoresis to separate various-sized DNA pieces in a sample.

- [GeneReviews](#)

This website allows users to search for peer-reviewed, expert information on a specific genetic condition.

- [GeneTestsEdit](#)

The GeneTests website offers an outstanding series of expert-authored GeneReviews that provide important information for clinicians to know about diagnosis, natural history, and genetic testing for genetic conditions. GeneTests.org also maintains databases of genetic testing laboratories and medical genetics clinics. There is no cost to use this website.

- [Karyotyping](#) 

Morphological evaluation of chromosomes is done by creating a picture, called a karyotype, of the chromosomes in a single cell. A karyotype can be created from a variety of samples, including blood, bone marrow, amniotic fluid, or placental tissue. Each sample is cultured in the laboratory; when ready, the cells are harvested. The chromosomes they contain are then stained, viewed under a microscope, and arranged into 23 pairs.

- [National Newborn Screening and Genetics Resource Center \(NNSGRC\)](#)

This website provides information for both families and health care professionals on newborn screening and genetics.

- [National Newborn Screening Status Report](#)

This document lists the screening tests performed on newborns by state and notes whether they are required by law or not.

- [Polymerase Chain Reaction](#) 

A sample of amniotic fluid yields a small amount of cells and, therefore, DNA. Often when performing genetic testing on amniotic fluid, the first step is to use a technique known as polymerase chain reaction (or PCR) to make many copies of the region of DNA that is of interest, since there are too few to study in the original amniotic fluid sample. This process is called amplification and can be done on DNA extracted from any tissue type. In the case of sickle cell anemia, the beta-globin gene is amplified.

- [Protein Separation](#) 

Proteins, the products of genes, vary from one another by shape, size, charge, molecular affinity, and other characteristics. Proteins made from different genes usually differ from each other in significant ways. However, proteins made by the same gene may also exhibit different characteristics. These differences can be the result of the presence of a genetic mutation. Such differences can be used to identify the presence of normal and abnormal proteins.

- [Protein Truncation Testing](#) 

For mutations that commonly cause shortened (truncated) protein products, protein truncation testing (PTT) can be very useful. In the lab, proteins are artificially synthesized from the gene in question. The protein products are then separated by size on a special gel. A smaller-than-normal protein on the gel indicates the presence of a truncating mutation.

- [Restriction Enzyme Digestion](#) 

Restriction enzyme digestion describes the process used to cut, or digest, large pieces of DNA into smaller pieces. DNA is cut by combining it with a special type of enzyme, a restriction

enzyme, which "recognizes" a unique DNA sequence. The mixture is then incubated under laboratory conditions to maximize digestion. During digestion, the enzyme locates places along the DNA with the unique sequence and cuts the DNA at that site.

- **Southern Blotting** 

Southern blot analysis involves transferring DNA fragments embedded within a gel (after electrophoresis) onto a special membrane. Because the fragments transferred onto the membrane are too small to be seen, probes created to target a specific DNA sequence must be added. Probes are relatively short stretches of DNA that are tagged, often with fluorescence or radioactivity, allowing them to be visualized when bound to the DNA on the membrane. Probes will only bind or stick to the specific piece of DNA for which they are designed.

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