Technical standards and guidelines for reproductive screening in the Ashkenazi Jewish population

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Disclaimer: *These Technical Standards and Guidelines were developed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory genetic services. Adherence to these standards and guidelines is voluntary and does not necessarily assure a successful medical outcome. These Standards and Guidelines should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the clinical laboratory geneticist should apply his or her own professional judgment to the specific circumstances presented by the individual patient or specimen.*

Clinical laboratory geneticists are encouraged to document in the patient's record the rationale for the use of a particular procedure or test, whether or not it is in conformance with these Standards and Guidelines. They also are advised to take notice of the date any particular standard or guidelines was adopted, and to consider other relevant medical and scientific information that becomes available after that date. ***Genet Med*** **2008:10(1):57–72.**

The Ashkenazi Jewish population represents a distinct subpopulation characterized by a specific religion (Judaism), a defined place of origin (Middle East) and a well-defined pattern of migration (eastern Europe, United States). Traditionally, Jews have been divided into three major groups according to their regions of residence: Ashkenazi Jews, Middle Eastern (or Oriental Jews), and Sephardic Jews.[1] Approximately 90% of the 5.7 million Jews in the United States are classified as Ashkenazi Jewish. Historically, this population has been interested in and willing to participate in genetic carrier testing programs, beginning over 30 years ago with the introduction of Tay-Sachs disease (TSD) enzymatic screening in the 1970s.

At least 40 genetic conditions with known inheritance patterns and molecular basis have been described in different Jewish groups.[1] In every disease, at least one common founder mutation is present, often along with additional less common mutations. For some conditions, such as cystic fibrosis (CF), many other mutations have been found in non-Jewish populations.[2] For others, such as familial dysautonomia (FD), the disorder almost exclusively occurs in the Ashkenazi Jewish population. Genetic testing is available for many common autosomal recessive conditions in the Ashkenazi Jewish population and is routinely offered as a panel for eight or more conditions by several laboratories.

Approximately 1 in 40 Ashkenazi Jewish individuals carry 1 of 3 common *BRCA1/2* mutations.[3] However, the penetrance of these mutations is not fully understood and adequate laboratory and clinical resources for performing the testing and genetic counseling are not currently available. Because of the complex issues concerning screening and genetic counseling, the American College of Medical Genetics (ACMG) does not support general Ashkenazi Jewish population screening for *BRCA* mutations, in the absence of an IRB-approved research protocol.[4]

Recently, the American College of Obstetricians and Gynecologists' Committee on Genetics recommended that couples of Ashkenazi Jewish ancestry be offered prenatal or preconception screening for the following disorders: TSD, Canavan disease (CD), CF, and FD. In addition, individuals of Ashkenazi Jewish descent "may inquire about the availability of carrier screening for other disorders, including mucolipidosis IV, Niemann-Pick disease type A, Fanconi anemia group C, Bloom syndrome and Gaucher disease."[5]

This document is not intended for use as a clinical practice guideline. The purpose of this manuscript is to present an overview of the prevalent disorders in the Ashkenazi Jewish population, which could potentially be included in a screening program, and then focus exclusively on the laboratory standards and guidelines for prenatal- and preconception-based carrier screening for TSD, CD, and FD. ACMG laboratory standards and guidelines that cover the Ashkenazi Jewish population for CF are available elsewhere.[6]

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TAY-SACHS DISEASE (TSD)

Gene Symbol/Chromosome Locus: *HEXA*; 15q23-q24
OMIM Number: 272800 (Tay-Sachs disease); 606869 (hexosaminidase A)

TSD is a devastating autosomal recessive lysosomal storage disease caused by a deficiency in the enzyme β-hexosaminidase A. Almost all patients present with an infantile form, with onset of symptoms in the first year of life and death by the age of 3 to 4 years with progressive neurologic deterioration; a rarer adult-onset form also occurs. The carrier frequency among Ashkenazi Jews is approximately 1 in 31.[7] Population-based screening programs have led to a 90% decrease in the incidence of TSD in the North American Jewish population. Carrier screening is also recommended for individuals of French Canadian and Cajun descent because of the high carrier frequency in those populations as well. Initially, carrier screening was based on the measurement of hexosaminidase A enzyme levels, with a 97% to 98% detection rate. Current molecular-based assays test for three common mutations (1278insTATC, G269S and 1421 + 1G→C), which will detect more than 98% of obligate Jewish carriers and 93% of Jewish carriers identified by enzymatic screening.[7] In a carrier screening program with this detection rate, approximately 95% of affected fetuses would be detected if a prenatal procedure was performed.

CANAVAN DISEASE (CD)

Gene Symbol/Chromosome Locus: *ASPA*; 17pter-p13
OMIM Number: 271900 (Canavan disease); 608034 (aspartoacylase)

Similar to TSD, CD is a devastating, fatal neurodegenerative leukodystrophy resulting from the deficiency of the enzyme aspartoacylase. Symptoms usually occur within the first few months of life and the disease is fatal in early childhood. The Ashkenazi Jewish carrier frequency is approximately 1 in 41 (Table 1) and carrier detection is based on the identification of two common mutations: E285A and Y231X, which account for approximately 97% to 98% of mutations in this population. A third mutation, A305E, accounts for ∼1% of mutations in the Ashkenazi Jewish population but approximately 50% of non-Jewish mutant alleles.[12] In a carrier screening program with a 97% to 98% detection rate, approximately 95% of affected fetuses would be detected if a prenatal procedure was performed.

Table 1
Observed and corrected carrier rates for Canavan disease among the Ashkenazi Jewish population

Study location	Mutations tested	Number	Carriers	Rate (corrected)
Israel[8]	E285A	879	15	58.6 (48.6)
New York, NY[9]	E285A and Y231X	449	11	40.8 (39.7)
United States[10]	E285A and Y231X	4,000	106	37.7 (36.8)
Toronto, Canada[11]	E285A, Y231X and A305E	1,423	25	56.9 (56.9)
All		6,751	157	42.9
			163.2[a]	**41.4**[a]

[a]After accounting for the number of mutations tested for by each study.

GAUCHER DISEASE (GD)

Gene Symbol/Chromosome Locus: *GBA*; 1q21
OMIM Number: 230800

GD is an autosomal recessive lysosomal storage disease resulting from a deficiency of the enzyme glucocerebrosidase. There are three types of GD. Type I (nonneuropathic form) is the most common in the Ashkenazi Jewish population. The disorder is clinically heterogeneous, with symptoms ranging from onset of severe disease in childhood/adolescence to a mild disease with no symptoms or initial symptoms beginning in adulthood. The carrier frequency among Ashkenazi Jews is approximately 1:18.[13] Carrier testing in the Ashkenazi Jewish population is based on the analysis of three (or four) common mutations: N370S, 84GG, L444P (and IVS2 + 1G→A), which account for approximately 95% of the Ashkenazi Jewish mutations. In a carrier screening program with this detection rate, approximately 90% of affected fetuses would be detected if a prenatal procedure was performed. Type I patients who are homozygous for the N370S mutation are less severely affected and develop symptoms later in life. Enzyme replacement therapy is available for patients affected with GD. Because of the highly variable expression of this condition, some concern has been raised about including GD in preconception- and prenatal-screening programs.[14,15]

CYSTIC FIBROSIS (CF)

Gene Symbol/Chromosome Locus: *CFTR*; 7q31.2
OMIM Number: 219700 (CF); 602421 (Cystic Fibrosis Transmembrane Conductance Regulator)

Testing for a limited number of mutations (included in the current ACMG-recommended screening panel) will identify 94% of the CF mutations in the Ashkenazi Jewish population. Refer to laboratory standards and guidelines for CF, available online.[6]

FAMILIAL DYSAUTONOMIA (FD)

Gene Symbol/Chromosome Locus: *IKBKAP*; 9q31
OMIM Number: 223900 (Neuropathy, Hereditary sensory and autonomic, Type III: HSAN-III); 603722 (Inhibitor of kappa light polypeptide gene enhancer in B cells, Kinase complex-Associated protein; IKBKAP)

FD is an autosomal recessive neuropathy that almost exclusively occurs in the Ashkenazi Jewish population. Also known as Riley-Day syndrome, the disorder is characterized by episodes of vomiting, decreased sensitivity to pain and temperature, and cardiovascular instability. The autonomic crises are life-threatening; age of onset is usually during infancy. The carrier frequency is approximately 1 in 31. A single mutation in

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intron 20 of the *IKBKAP* gene (2507 + 6 T→C) accounts for >99% of the mutations in the Ashkenazi Jewish population.[16,17] A second mutation, R696P, is included in most carrier screening panels and accounts for the remainder of Ashkenazi Jewish mutations. In a carrier screening program with this detection rate, at least 99% of affected fetuses would be detected if a prenatal procedure was performed.

NIEMANN-PICK DISEASE, TYPE A (NPD-A)

Gene Symbol/Chromosome Locus: *SMPD1*; 11p15.4-p15.1
OMIM Number: 257200

Niemann-Pick disease, type A, is an autosomal recessive lysosomal storage disease resulting from a deficiency of the enzyme sphingomyelinase. Type A disease is a rapidly progressive neurodegenerative disease that begins during infancy and is fatal by 3 to 5 years of age. The carrier frequency in the Ashkenazi Jewish population is approximately 1 in 90.[18] The common mutations fsP330, R496L, and L302P account for approximately 97% of the Ashkenazi Jewish mutations.[18] In a carrier screening program with this detection rate, approximately 94% of affected fetuses would be detected if a prenatal procedure was performed. In addition, clinical testing for the Niemann-Pick, type B mutation, delR608 (which is not limited to the Ashkenazi Jewish population), is also available.

BLOOM SYNDROME

Gene Symbol/Chromosome Locus: *BLM*; 15q26.1
OMIM Number: 210900 (Bloom Syndrome); 604610 (RECQ Protein-like 3; RECQL3)

Bloom syndrome is an autosomal recessive disorder that is caused by mutations in the *BLM* gene, a RecQ helicase,[19] and is characterized by severe pre- and postnatal growth deficiency, skin sensitivity to sunlight, immunodeficiency and a predisposition to cancer. The carrier frequency in the Ashkenazi Jewish population is approximately 1 in 107 and a single mutation (BLM^{Ash}, a 6-bp deletion and 7-bp insertion: 2281del6/ins7; delATCTGA 2281insTAGATTC) accounts for >99% of the mutations in this population.[20] In a carrier screening program with this detection rate, at least 99% of affected fetuses would be detected if a prenatal procedure was performed.

MUCOLIPIDOSIS IV (MLIV)

Gene Symbol/Chromosome Locus: *MCOLN1*; 19p13.3-p13.2
OMIM Number: 252650 (MLIV); 605248 (Mucolipin 1; MCOLN1)

Mucolipidosis IV is a neurodegenerative lysosomal storage disorder characterized by poor growth, severe mental retardation, and ophthalmologic abnormalities (corneal clouding and progressive retinal degeneration). Lifespan may be normal, though developmental delays are severe. The carrier frequency in the Ashkenazi Jewish population is approximately 1 in 127.[21] Two mutations, IVS3-2A→G and del6.4kb, account for approximately 95% of the Ashkenazi Jewish mutations. In a carrier screening program with this detection rate, approximately 90% of affected fetuses would be detected if a prenatal procedure was performed.

FANCONI ANEMIA GROUP C (FA-C)

Gene Symbol/Chromosome Locus: *FANCC*; 9q22.3
OMIM Number: 227645

FAC is an inherited anemia that progresses to pancytopenia with increased risk for myelodysplastic syndrome, acute leukemia and solid tumors. The disorder is characterized by variable phenotypic findings that can include short stature, developmental delay, congenital anomalies of the limbs, heart and/or kidney. The carrier frequency in the Ashkenazi Jewish population is approximately 1 in 89, and a single mutation in the *FAC* gene IVS4 + 4A→T accounts for at least 99% of the mutations in this population.[22] In a carrier screening program with this detection rate, at least 98% of affected fetuses would be detected if a prenatal procedure was performed.

CANAVAN DISEASE-BACKGROUND

Gene Symbol/Chromosome Locus: *ASPA*; 17pter-p13
OMIM Number: 271900 (Canavan disease); 608034 (aspartoacylase)

Brief clinical description

CD is a progressive neurodegenerative disorder, caused by a deficiency of the enzyme aspartoacylase. It is most prevalent among Ashkenazi Jewish individuals, but has been reported in many ethnic and racial groups. The prevalence of CD in the Ashkenazi Jewish population is estimated to be about 1 in 9,100. The corresponding consensus carrier frequency in this population is 1 in 48, but there is some unexplained heterogeneity. The onset of symptoms is usually noted 3 to 6 months after birth. Clinical features include macrocephaly, hypotonia, head lag, and developmental delay. As the disease progresses, hypertonia develops. Other symptoms include: irritability, optic atrophy, sleep disturbance, seizures and feeding difficulties. Severity and life expectancy vary, with some children dying in the first year of life and others surviving into their teens. Treatment for CD is supportive and includes improvement of nutrition and hydration, treating seizures, managing infections, and protecting the airway. Some children benefit from physical therapy, early intervention, and special education. For more information see the online GeneClinics profile.[23] CD is diagnosed by high urine concentrations of *N*-acetyl aspartic acid (NAA). Molecular genetic testing can be used for clinical confirmation, carrier testing, and prenatal testing.

Mode of Inheritance: Autosomal recessive

Gene Description/Normal Gene Product

ASPA was cloned in 1993 and consists of 29kb with six exons and five introns. The normal gene product is 313 amino acids with a mass of 36 kDa. ASPA hydrolyzes NAA into aspartic acid

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Table 2
Common Canavan disease mutations in the Ashkenazi Jewish population

Nucleotide change	Amino acid change	Type	No. (%) of alleles identified among 96 affected Ashkenazi Jewish individuals[8,12]
854 A→C	E285A	Missense	160 (83.3)
693 C→A	Y231X	Nonsense	27 (14.1)
914 C→A	A305E	Missense	2 (1.0)
433-2 A→G	Not applicable	Splicing	1 (0.5)
Other			2 (1.0)

and acetate. The ASPA transcript and protein have a wide tissue distribution.[24,25]

Mutational mechanism/abnormal gene product

ASPA mutations include null mutations, which produce no enzyme, and missense mutations which produce aspartoacylase with decreased activity. Although the enzyme is widely expressed, its absence or decreased activity results in accumulation of NAA in the brain. This leads to neurodegeneration and disease symptoms.

Mutation spectrum

A complete list of ASPA mutations can be found in the Human Gene Mutation Database.[26] More than 50 different mutations associated with CD have been described. Most of these mutations are rare and unique to specific families. The common mutations are listed in Table 2. Two mutations E285A (854 A→C) and Y231X (693 C→A) account for 97.4% (95% CI 94.0% to 99.1%) of Ashkenazi Jewish CD alleles, but only a small portion (3%) of non-Jewish mutations. One additional mutation, 433-2 A→G, accounts for an additional 0.5% of Ashkenazi Jewish mutations. A305E (914 C→A) accounts for 40% to 60% of non-Jewish CD mutations but only 1% of Ashkenazi Jewish mutations. Of these mutations, only E285A has residual (2.5%) ASPA activity. The other common mutations lead to a complete loss of enzyme activity in vitro.

Genotype–phenotype considerations

Regardless of ethnicity, the phenotype of individuals homozygous for mutations associated with a complete lack of enzyme activity are phenotypically indistinguishable from patients who are homozygous for E285A, associated with some residual activity. However, the phenotype is variable, even among patients with the same genotype, although all are eventually symptomatic. Although a few mutations associated with a more severe phenotype have been reported, they are too rare to make accurate genotype–phenotype correlations.

Alternative testing methods

NAA can be measured in the urine, blood and cerebrospinal fluid by gas chromatography-mass spectrometry. Urine NAA concentration is sufficient for diagnosing affected individuals. When familial mutations cannot be identified, prenatal testing for at-risk fetuses can be performed between 16 and 18 weeks by assaying the concentration of NAA in amniotic fluid. Cultured amniocytes or chorionic villi cannot be used for this assay.

Aspartoacylase enzymatic activity is usually undetectable in skin fibroblasts from affected individuals. Heterozygous carriers have approximately 50% of the normal level of enzyme activity. This enzyme cannot be assayed from a blood specimen or be used for prenatal diagnosis. Aspartoacylase enzyme activity is generally not used for carrier or diagnostic testing in clinical laboratories.

Clinical validation: clinical sensitivity and specificity

Clinical sensitivity

Clinical sensitivity is defined as the proportion of couples where both members carry a CD mutation (or Ashkenazi Jewish pregnancies with CD) that is detectable using a DNA test for selected mutations. If testing is performed for the two most common mutations (representing 97.4% of the mutations), then 94.9% of carrier couples (or affected pregnancies) could be detected (95% CI 88.4–98.2%). This makes the assumption that among carrier couples, amniocentesis and genotyping of the fetus is diagnostic.

These estimates assume that the analytic sensitivity of the assay is 100% (i.e., no false negative results occur) and this is unlikely. Data from large scale proficiency testing suggest that analytic sensitivity could be as low as 98% in some cases.[27,28] That is, in 2% of carriers the laboratory test produces a false negative test result. The resulting clinical sensitivity estimates are lowered to 91% (two mutations) and 94% (three mutations).

Clinical specificity

Clinical specificity can be defined as the proportion of noncarrier couples that are correctly identified as noncarrier couples. Assuming that testing is sequential (the female is tested first, with the male tested only if his partner is found to be a carrier), a false positive couple could occur if the woman is a true positive and the man is a false positive or if the woman is a false positive and the man is a true positive. No data yet exist to document the analytic false positive rate, but general laboratory experience suggests that occasional false positive results would be expected to occur.

Prevalence

Prevalence can be defined as the proportion of livebirths affected with CD in the absence of prenatal diagnosis and selective termination. Prevalence can be estimated directly by using newborn registries or indirectly using carrier rates. There are reliable data for CD carrier rates in the Ashkenazi Jewish population, and these are shown in Table 1. Assuming a carrier rate of 1 in 41.4 (95% CI 1 in 35.8 to 1 in 49.0), the corresponding birth prevalence would be 1 in 6856 (95% CI 1 in 5127 to 1 in 9604).

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Clinical positive predictive value

Clinical positive predictive value is defined as the probability that a positive test result (e.g., an Ashkenazi Jewish carrier couple for CD) is correct. This value can be computed by knowing the analytic and clinical sensitivity and specificity as well as prevalence of the disorder. The common CD mutations will produce a CD clinical phenotype so the clinical positive predictive value will be high (most carrier couples will have a 25% reproductive risk). Exceptions will occur, however, because of analytic false positives. Because of this possibility, clinical laboratories should confirm the carrier couple status prior to or as part of prenatal diagnostic testing.

Clinical negative predictive value

Clinical negative predictive value is defined as the probability that a negative test result (e.g., an individual's carrier result, or the couple's carrier result) is correct. Assuming analytic sensitivity and specificity of 100%, a false negative result will occur because the mutation present is not being tested for by the laboratory. The best estimate is that 2.6% (testing for two most common mutations) or 1.6% (testing for three most common mutations) of mutations will not be identified. Among individuals with an initial carrier risk of 1 in 41, testing negative for two mutations reduces the risk of being a carrier to 1 in 1540; testing for three mutations reduces the risk of being a carrier to 1 in about 2,504. If the female tests positive and the male tests negative, then the residual risk of having an affected pregnancy is 1 in 6,160 (two mutations) or 1 in 10,014 (three mutations). If analytic validity is $<$100%, then all estimates will be somewhat higher.

TAY-SACHS DISEASE-BACKGROUND

Gene Symbol/Chromosome Locus: *HEXA*; 15q23-q24
OMIM Number: 272800 (Tay-Sachs disease); 606869 (hexosaminidase A)

Brief clinical description

TSD is a progressive neurodegenerative disorder, caused by a deficiency of the enzyme β-hexosaminidase A. TSD occurs across all ethnic groups and races, with a general population carrier frequency of about 1 in 300. TSD is especially prevalent among Ashkenazi Jews, French Canadians, Cajuns, and the Old Order Amish in Pennsylvania. The carrier frequency for TSD among these groups is approximately 10-fold higher than in the general population or about 1 in 31. Before the implementation of Ashkenazi Jewish population-based carrier screening in 1970, the disease incidence was about in 1 in 3800 in this group. Children with TSD develop normally for the first few months of life and then begin to exhibit severe mental and physical deterioration. TSD is characterized by progressive weakness and loss of motor skills, decreased attentiveness and increased startle response. A macular cherry red spot is typical. As the disease progresses, seizures and blindness develop. Children with TSD usually do not live past 3 to 5 years of age. There are milder variants of hexosaminidase A deficiency, with juvenile (chronic) and adult-onset forms. These variants have later onset with slower disease progression. Treatment for TSD is supportive and includes improvement of nutrition and hydration, treating seizures, managing infections and protecting the airway. Enzyme replacement therapy and bone marrow transplant are ineffective. Substrate reduction therapy using miglustat has been proposed as a potential therapy for several lysosomal storage disorders, including infantile TSD.[29] For more information see the online GeneClinics profile.[23] TSD is diagnosed by deficient activity of hexosaminidase A in serum or white blood cells. In Ashkenazi Jewish individuals, molecular genetic testing can be used for clinical confirmation in affected individuals, confirmation of the carrier state (to exclude the existence of a pseudodeficiency allele), carrier testing and prenatal testing.

Mode of Inheritance: Autosomal recessive

Gene Description/Normal Gene Product

HEXA was isolated in 1987 and consists of 35 kb with 14 exons, a 5′ regulatory element and 3′ untranslated region. The normal gene product is 529 amino acids with a mass of 61 kDa. *HEXA* encodes the alpha subunit of the β-hexosaminidase A (HEX A) enzyme. The HEX A enzyme is a dimer composed of the alpha subunit coded for by *HEXA* and a beta subunit coded for by the *HEXB* gene (5q13). HEX A functions to hydrolyze the sphingolipid GM2 ganglioside.

Mutational mechanism/abnormal gene product

HEXA mutations include insertions, deletions, splice site, nonsense, and missense mutations. These mutations affect enzyme processing, assembly or activity. Decreased or absent HEX A activity leads to lysosomal accumulation of GM2 ganglioside, especially in the nervous system.

Mutation spectrum

Over 95 mutations associated with TSD have been reported, the majority are associated with the acute infantile form of the disease. A complete list of *HEXA* mutations associated with TSD can be found in the Human Gene Mutation Database[26] and the Human Genetic Disease Database.[30] Three common deleterious mutations (Table 3) account for 98.8% of muta-

Table 3
Common Tay-Sachs disease mutations among Ashkenazi Jewish obligate carriers

Nucleotide change	Affect on DNA or amino acid	Type	No. (%) of alleles identified among 87 obligate carriers[31]
1278insTATC	4 base insertion in exon 11	Frameshift	71 (81.6)
1421 + 1G→C (in IVS 12)	G→C at the intron 12 5′ splice site	Splicing	10 (11.5)
805 G→A (exon 7)	G269S	Missense	5 (5.8)
	Other		1 (1.1)

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tions in the Ashkenazi Jewish population.[31] Two mutations account for 46% of non-Jewish mutations, 1278insTATC and 1073 + 1G→A (in IVS 9). The majority of mutations result in null alleles, associated with the complete loss of enzyme activity. G269S affects the alpha subunit's ability to adopt the proper conformation, leading to its inability to dimerize with the beta subunit. Other mutations unique to specific populations have been reported. For example, a 7.6 kb deletion of the 5′ end of *HEXA* accounts for ~70% of the mutations among French Canadians.

Pseudodeficiency alleles

Two alleles, R247W and R249W are associated with HEX A pseudodeficiency. These pseudodeficiency alleles are associated with reduced HEX A enzyme activity toward the artificial substrate used in the biochemical screening method. Therefore individuals with these alleles appear to be TSD carriers based on enzyme analysis. However, these alleles do not affect the in vivo enzyme activity against GM2 ganglioside and are therefore not associated with disease. Individuals with one pseudodeficient allele and one mutant allele have very low or no in vitro HEX A enzyme activity, but no evidence of disease. Confirmation of carrier status using DNA-based testing can exclude pseudodeficiency in an individual with a positive enzyme-based screening result. R247W is found in 2% of Jewish and 32% of non-Jewish individuals identified as carriers through the biochemical assay. Four percent of non-Jewish individuals identified as carriers by enzyme-based screening carry the R249W allele.

Genotype–phenotype considerations

Individuals with classic TSD have two severe alleles with absent HEX A activity. Individuals with later onset forms (juvenile or chronic and adult) are usually compound heterozygotes for a severe allele and a milder mutation or have two mild alleles. The vast majority of *HEXA* mutations, including 1278insTATC and 1421 + 1G→C are associated with absent HEX A activity. G269S is a milder mutation, common among patients with late-onset TSD.[32] Therefore, DNA testing can provide important genotype–phenotype information used in genetic counseling for at-risk couples.

Alternative testing methods

Hexosaminidase A activity assays can be used for carrier screening and diagnostic testing for TSD. This is a simple, inexpensive and very accurate method to determine carrier status. Affected individuals have absent to near-absent enzyme activity in serum, platelets and leukocytes.[33,34] Serum HEX A screening can be used in males and females who are not pregnant or taking oral contraceptives. Leukocyte or platelet screening must be used in women who are pregnant, using oral contraceptives, or individuals of either sex with an inconclusive serum HEX A result.[35] One study supports the use of DNA-based testing for carrier screening in individuals of full Ashkenazi Jewish ancestry.[36] Biochemical screening should be used for non-Jewish individuals[37] and for patients of mixed ancestry (i.e., those with <4 Ashkenazi Jewish grandparents). TSD carriers identified with HEX A screening should be offered confirmatory DNA studies to exclude the presence of a pseudodeficiency allele.

For prenatal testing, when both parents are known carriers and the possibility of a pseudodeficient allele is excluded, HEX A enzymatic activity can be used for testing fetal cells obtained by chorionic villus sampling or amniocentesis. However, if the parental mutations are known, then DNA-based testing should be performed on fetal cells.

Clinical validation: clinical sensitivity and specificity

Clinical sensitivity

If the three most common mutations were tested for by the laboratory (representing 98.9% of the mutations), then 97.8% of carrier couples (or affected pregnancies) could be detected (95% CI 93.8–99.9%). This makes the assumption that among carrier couples, amniocentesis, and genotyping of the fetus is diagnostic.

These estimates assume that the analytic sensitivity of the assay is 100% (i.e., no false negative results occur) and this is unlikely. Data from large scale proficiency testing suggest that analytic sensitivity could be as low as 98% in some cases.[27,28] That is, in 2% of carriers the laboratory test produces a false negative test result. The resulting clinical sensitivity estimates are lowered to 95%.

Clinical specificity

Assuming that testing is sequential, a false positive couple will most likely occur when the female is a true positive and the male is a false positive. No data yet exist to document the analytic false positive rate, but general laboratory experience suggests that occasional false positive results would be expected to occur.

Prevalence

There are reliable data for TSD carrier rates in the Ashkenazi Jewish population, and these are shown in Table 4. Assuming a carrier rate of 1 in 31.0 (95% CI 1 in 29.9 to 1 in 32.2), the corresponding birth prevalence would be 1 in 3800 (95% CI 1 in 3600 to 1 in 4200).

Clinical positive predictive value

The three common TSD mutations will produce a clinical phenotype so the clinical positive predictive value will be high (most carrier couples will have a 25% reproductive risk). Exceptions will occur, however, because of analytic false posi-

Table 4
Observed carrier rates for Tay-Sachs disease among the Ashkenazi Jewish population

Study location	Mutations tested	Number	Carriers	Rate
International[7]	1278insTATC, 1421 + 1G→C, G269S, IVS9 + 1G→A	91,217	2,946	31.0

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tives. Because of this possibility, clinical laboratories should confirm the carrier couple status prior to or as part of prenatal diagnostic testing.

Clinical negative predictive value

Assuming analytic sensitivity and specificity of 100%, a false negative result will occur because the mutation is not being tested for by the laboratory. The best estimate is that 1.1% of mutations will not be identified. Among individuals with an initial carrier risk of 1 in 31.0, testing negative for the three common mutations reduces the risk of being a carrier to 1 in 2,700. If the woman tests positive and the man tests negative, the residual risk of having an affected pregnancy is about 1 in 11,000. If analytic validity is <100%, then all estimates will be somewhat higher.

FAMILIAL DYSAUTONOMIA BACKGROUND

Gene Symbol/Chromosome Locus: *IKBKAP*; 9q31

OMIM Number: 223900 (Neuropathy, Hereditary sensory and autonomic, Type III: HSAN-III); 603722 (Inhibitor of kappa light polypeptide gene enhancer in B cells, Kinase complex-Associated protein; IKBKAP)

Brief clinical description

FD, also known as Riley-Day syndrome or hereditary sensory neuropathy Type III (HSN-III) is characterized by extensive sensory dysfunction and is the most common and widely recognized of the congenital sensory neuropathies.[38–40] FD affects the development and survival of sensory, sympathetic, and parasympathetic neurons. It is present from birth and is debilitating. Before the underlying gene defect was described the diagnosis of FD was based on the following cardinal criteria: absence of fungiform papillae on the tongue; absence of axon flare after injection of intradermal histamine; decreased or absent deep tendon reflexes; and absence of overflow emotional tears. Approximately 40% of individuals have autonomic crises. Hypotonia contributes to delay in acquisition of motor milestones. Life expectancy is significantly less than normal.[23,41] The development and survival of sensory, sympathetic, and parasympathetic neurons are affected and neuronal degeneration continues throughout life. Affected individuals have gastrointestinal dysfunction, vomiting crises, recurrent pneumonia, altered sensitivity to pain and temperature, and cardiovascular instability.[38,42–46] Axelrod et al[47] demonstrated that improved supportive treatment extended the survival of individuals with FD and that the probability of reaching 20 years of age has increased to 60%. Fertility in both males and females with FD has been proven.[48]

The incidence of FD is 1 per 3900 births with a carrier frequency of about 1 in 31 in the Ashkenazi population.[16,17,49–51] A different carrier frequency was found among a subset of Polish Ashkenazi Jews: 11 carriers among 195 individuals (1 in 18) in contrast to only 3 in 298 (1 in 100) of full non-Polish background. Only one non-Jewish individual has been found with a mutation in *IKBAKAP*.[52]

Mode of inheritance: Autosomal recessive

Gene Description/Normal Gene Product

Mutations in *IKBKAP* are known to be associated with FD. The gene codes for the protein IkappaB kinase complex-associated protein. The *IKBKAP* gene was cloned in 2001 and consists of 37 exons and encodes a protein of 1332 amino acids. The protein is called IKAP.[16,17] Northern blot analysis of *IKBKAP* reveals two mRNAs of 4.8 and 5.9 kb. The 5.9 kb message differs only in the length of the 3′ untranslated region and is predicted to encode an identical 150 kDa protein. The IKAP protein is homologous to the Elp1 protein of *Saccharomyces cerevisiae,* which is a member of the six subunit Elongator complex. The complex is associated with hyperphosphorylated RNA polymerase II during transcriptional elongation in yeast. The functional complex, termed "holo-Elongater," is unstable and can dissociate into two discrete three-subunit complexes. One member of the complex, Elp3, is a highly conserved histone acetyltransferase (HAT), and HAT activity suggests that Elongator is involved in creating a chromatin structure that permits efficient elongation of mRNA during transcription. Very recently, the human Elongator complex has been purified as a six-subunit complex and shown to contain IKAP, hELP3, StIP1, hELP4 and two additional unidentified proteins. Homology between the yeast and human proteins is extensive. IKAP is found primarily in the nucleus, however it was also shown to be in the nucleoli and cytoplasm by immunostaining.

Mutational mechanism/abnormal gene product

One mutation represents nearly 99% of the mutations. This is a T→C transition mutation at base pair 6 of the *IKBKAP* intron 20 splice donor site. Careful examination of mRNA from different tissues and cell lines of affected individuals demonstrates that both wild-type and mutant mRNA is expressed.[53] The R696P mutation is predicted to disrupt a potential phosphorylation site at residue 696.

Mutation spectrum

Table 5 shows the two FD mutations reported in the Ashkenazi Jewish population, 2507 + 6T→C and R696P (G→C transversion at base pair 2397 in exon 19), representing 99.4% (95% CI 98.1–99.9%) of the mutations. These mutations are also listed in the Human Gene Mutation Database.[26] A third

Table 5

Common familial dysautonomia disease mutations in the Ashkenazi Jewish population

Nucleotide change	Affect on DNA or amino acid	Type	No. (%) of alleles identified among 236 affected Ashkenazi Jewish individuals[16,49]
2507 + 6T→C (IVS 20)	T→C at the intron 20 3′ splice site	Splicing	466 (98.7)
2397 G→C	R696P	Missense	3 (0.7)
Other			3 (0.7)

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mutation, 3051 C→T (P914L), has been described in one family that was not of Ashkenazi Jewish descent.

Genotype–phenotype considerations

There are no known genotype–phenotype correlations in FD since one sequence change accounts for nearly 99% of the reported mutations.

Clinical validation: clinical sensitivity and specificity

Clinical sensitivity

If the two most common mutations are utilized (representing 99.4% of the mutations), then 98.7% of carrier couples (or affected pregnancies) could be detected (95% CI 96.2–99.9%). This makes the assumption that among carrier couples, amniocentesis and genotyping of the fetus is diagnostic.

These estimates assume that the analytic sensitivity of the assay is 100% (i.e., no false negative results occur) and this is unlikely. Data from large scale proficiency testing suggest that analytic sensitivity could be as low as 98% in some cases.[27,28] That is, in 2% of carriers the laboratory test produces a false negative test result. The resulting estimate of clinical sensitivity for FD is lowered to 95% (two mutations).

Clinical specificity

Assuming that testing is sequential, a false positive couple will most likely occur when the female is a true positive and the male is a false positive. No data yet exist to document the analytic false positive rate, but general laboratory experience suggests that occasional false positive results would be expected to occur.

Prevalence

There are reliable data for FD carrier rates in the Ashkenazi Jewish population, and these are shown in Table 6. Assuming a carrier rate of 1 in 31.3 (95% CI 1 in 36.6 to 1 in 27.0), the corresponding birth prevalence would be 1 in 3900 (95% CI 1 in 2900 to 1 in 5400).

Table 6
Observed carrier rates for familial dysautonomia among the Ashkenazi Jewish population

Study location	Mutations tested	Number	Carriers	Rate (1 in N)
United States/Israel[49]	2507 + 6T→C	162	5	32.4
United States/Israel[17]	2507 + 6T→C and R696P	506	15	33.7
United States[16]	2507 + 6T→C and R696P	819	29	28.2
New York, NY[50]	2507 + 6T→C and R696P	2518	80	31.5
Israel[51]	2507 + 6T→C	1100	34	32.4
All		5105	163	31.3

Clinical positive predictive value

The two common FD mutations will produce a clinical phenotype so the clinical positive predictive value will be high (most carrier couples will have a 25% reproductive risk). Exceptions will occur, however, because of analytic false positives. Because of this possibility, clinical laboratories should confirm the carrier couple status prior to or as part of prenatal diagnostic testing.

Clinical negative predictive value

Assuming analytic sensitivity and specificity of 100%, a false negative result will occur because the mutation is not being tested for in the laboratory. The best estimate is that 0.6% of mutations will not be identified. Among individuals with an initial carrier risk of 1 in 31.3, testing negative for the two common mutations reduces the risk of being a carrier to 1 in 4900. If the female tests positive and the male tests negative, the residual risk of having an affected pregnancy is 1 in 9900. If analytic validity is < 100%, then all estimates will be somewhat higher.

SPECIAL TESTING CONSIDERATIONS

Indications for testing

- Diagnostic testing, possible diagnosis
- Diagnostic testing, definite diagnosis
- Carrier testing, positive family history
- Carrier testing, partners of individuals with positive family history
- Carrier testing, preconception screening for individuals of reproductive age
- Carrier testing, premarital population, to assist in selection of a spouse
- Carrier testing, prenatal screening
- Carrier testing, gamete donors
- Preimplantation genetic diagnosis
- Prenatal diagnostic testing, for couples wherein both partners are carriers
- Prenatal diagnostic testing, for women who are known carriers and their partners are unavailable for testing

Diagnostic versus carrier testing

The tests described above are primarily used for carrier testing in the Ashkenazi Jewish population. The identification of a single mutation in a carrier screen would be considered positive for that disorder. A negative result significantly decreases (but does not eliminate completely) the likelihood that an individual is a carrier for these disorders. A Bayesian calculation should be performed to determine an individual's remaining carrier risk. Occasionally, such as in GD, an asymptomatic patient may be identified as being homozygous. Preconception genetic counseling must include a discussion of that possibility.

In an Ashkenazi Jewish patient suspected of having one of the above disorders, molecular testing is highly accurate and

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the detection of two mutations is diagnostic. The identification of a single mutation in a suspected patient would be supportive of that diagnosis, but would not in itself be diagnostic. Additional studies (DNA sequencing or enzymatic analysis) would be necessary to confirm the diagnosis in those situations.

It is also possible that an individual would test positive as a carrier for more than one of the diseases listed above. Using disease carrier frequencies, it is estimated that about 1 in 4 to 1 in 5 Ashkenazi Jewish individuals is a carrier for at least one disorder. One study (which included individuals of non-Jewish and mixed Jewish ancestry) showed that 1 in 121 individuals tested for CF, TSD, GD, CD, NPD-A, Bloom syndrome, FD and FA-C were carriers of two diseases and 1 in 7 were carriers of a single disease.[54]

Prenatal testing

Prenatal diagnostic testing using DNA extracted from amniotic fluid or chorionic villus samples is indicated when both parents have an identifiable mutation for the same disorder. DNA samples should be obtained from both parents and run simultaneously with the prenatal sample to serve as controls for both the patient (i.e., the fetus) and the assay. When both parents are obligate carriers for CD or TSD, but the mutations are not identifiable using the available methods, prenatal testing can be performed by alternative methods (refer to sections "Alternative testing methods" and "Clinical validation: clinical sensitivity and specificity"). Couples should be referred for genetic counseling prior to any diagnostic procedure. For additional information, refer to Standards and Guidelines for Clinical Genetics Laboratories, Section G19.[55]

Maternal cell contamination

Refer to Standards and Guidelines for Clinical Genetics Laboratories, Section G19.3.[55]

GUIDELINES

Pretest considerations

Informed consent is required for preconception and prenatal carrier screening. Although it is generally the responsibility of the ordering health care professional (not the laboratory) to obtain consent, some states may have specific requirements that should be followed.

Although a laboratory may offer an Ashkenazi Jewish preconception/prenatal carrier screening panel that includes multiple diseases, the ordering physician might request testing for a specific disease(s) as opposed to the entire panel. To accommodate these requests, laboratories may wish to either set up individual tests (in addition to the panel) or send samples to another laboratory for an individual test. Factors that the ordering physician should take into consideration when deciding which tests to request include: disease prevalence, penetrance, age of onset, disease severity, treatment options, life expectancy, reproductive options, mutation detection rate, and cost. It is the laboratory's responsibility to provide this information to the health care provider so that meaningful informed consent can be obtained. The laboratory may provide the ordering physician with written educational materials to assist the patient in making an informed decision regarding which tests he/she wants performed.

Pretest clinical information

Laboratories should have a mechanism to collect pretest clinical information including: patient date of birth, sex, indication for testing, racial/ethnic background, and specific family history of pertinent diseases. If there is a positive family history of a specific disease, the laboratory should determine if the familial mutation(s) is (are) known. Pretest information can be collected using a test requisition form or patient questionnaire. If the laboratory receives no or inadequate preanalytical information, the ordering health care provider should be contacted prior to processing the specimen. If the laboratory does not obtain this information, the written report should indicate that preanalytical information was not received and therefore could not be taken into consideration. The report should also include a carrier risk revision table that allows the ordering physician to interpret carrier studies.

Methodological considerations

All general guidelines for laboratory techniques and quality control discussed in the ACMG Standards and Guidelines for Clinical Genetics Laboratories apply.[55] There are several valid methods with different advantages and disadvantages. Three different commercially available analyte-specific reagents (ASRs) are described in this document.

Positive controls

Commercially available control material. Control material can be obtained from the NIGMS Human Genetic Cell Repository[56] as DNA and/or cell lines. Positive control material for CD (Y231X and E285A), TSD (1278insTATC, 1421 + 1G→C and G269S), and FD (2507 + 6T→C) is currently available. A synthetic supercontrol that includes a panel of 32 common Ashkenazi Jewish mutations is available for use with a liquid bead platform. Supercontrols are also available for CF.[6]

Residual clinical specimens as positive controls. If a laboratory identifies a particular mutation in a patient sample, the residual DNA remaining from the clinical test can be anonymized and used as a positive control in future assays (provided the patient has consented for reuse of his/her DNA). If positive controls are generated using PCR or whole genome amplification, it is important that the laboratory take precautions to avoid contamination of the patient assays with the control PCR product.

Number of controls to be used in a multiplex assay. Although it is ideal to run a positive control for each mutation included in the assay, this is not always practical, depending on the number of mutations being screened and the technology the laboratory is using. It is recommended that the laboratory validate all

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mutations prior to offering the test on a clinical basis. Then during routine testing, each run should include at least one positive control and that all positive controls are used on a rotating basis.

Sample preparation

Most assays are amenable to the use of DNA prepared from whole blood or buccal samples using a variety of extraction protocols. DNA from prenatal specimens (amniocytes or chorionic villi) should be highly purified in order to be of sufficient quality for additional testing that may be required (e.g., maternal cell contamination studies). Typically, 10 to 50 ng of patient DNA is adequate for a robust amplification reaction.

Validation of methods

Commercial ASRs or a laboratory-developed method can be used. Regardless of whether the assay is developed in-house or purchased, laboratories offering genetic tests on a clinical basis are regulated under the provisions of Clinical Laboratory Improvement Amendments (CLIA)'88. This requires in-house validation of test performance prior to reporting results. A checklist for validation of genetic tests and other guidelines is available from the State of New York Department of Health.[57] For additional information on test validation procedures, refer to the ACMG Standards and Guidelines for Clinical Genetic Laboratories, Section C8.[55]

METHODS

Multiplex assays for testing multiple genes

Liquid bead

Overview. Liquid bead arrays provide simple and high-throughput analysis of DNA polymorphisms with discrete detection of wild-type and mutant alleles in a complex genetic assay.[58–62] Commercially available bead-array platforms are available for the detection of mutations associated with the following diseases: CF, TSD, CD, FD, GD, FA-C, Niemann-Pick, Bloom syndrome and MLIV.[63] Bead-array platforms use either universal tags or allele specific capture probes that are covalently immobilized on spectrally distinct microspheres. Because microsphere sets can be distinguished by their spectral addresses, they can be combined, allowing as many as 100 analytes to be measured simultaneously in a single-reaction vessel. A third fluorochrome coupled to a reporter molecule quantifies the molecular interaction that has occurred at the microsphere surface. The microspheres, or beads, are dyed internally with one or more fluorophores, the ratio of which can be combined to make multiple bead sets. Capture probes are covalently attached to beads via a terminal amine modification. Bead arrays offer significant advantages over other array technologies in that hybridization occurs rapidly in a single tube, the testing volume scales to a microtiter plate, and unlike glass or membrane microarrays, bead solutions can be quality tested as individual components.

Multiplex PCR Amplification. All general guidelines for multiplex PCR amplification apply to liquid bead array-based detection. All commercial products use a single multiplex PCR with proprietary primers designed to accommodate the hybridization and detection system being used. Because liquid bead arrays work well with various front-end chemistries, including oligonucleotide ligation, allele-specific single base extension, ASO hybridization, and allele-specific primer extension (ASPE), the detection chemistry of the particular detection format can be incorporated into the PCR and/or subsequent amplification modification steps.

Hybridization and detection. One commercial platform uses biotin-modified PCR products that are hybridized to allele-specific capture probes on different beads. Another uses allele-specific primer extension of the PCR product such that "universal tags" are incorporated into the product for allele discrimination. The biotinylated PCR product or extended PCR product is then hybridized to either capture probes or "universal antitags," respectively, which are covalently bound to the beads. Both platforms use a reporter fluorophore, streptavidin-phycoerythrin, in or before the hybridization reaction. After hybridization, the modified amplicon is bound to a reporter substrate and transferred directly to a detection instrument without posthybridization purification. The sample genotype is assigned by comparing the relative hybridization signal between the wild-type and mutant alleles. The generation of electronic data facilitates the development of automated analysis software and database archiving. The reaction is analyzed for bead identity and associated hybridization signal intensity. Lasers interrogate hybridized microspheres individually as they pass, single file, in a rapidly flowing stream. Thousands of microspheres are interrogated per second, resulting in an analysis system capable of analyzing and reporting up to 100 different hybridization reactions in a single well of a 96-well plate in just a few seconds.

Visualization and interpretation of results. Output files generated during detection are automatically processed and made available in a report format through customized software. The software should allow for controlled access to data, patient reports, comments, and sample history. Electronic data output is archived into a database format for data integrity, quality control tracking, and result trending and incorporates batch processing of results, highlighting samples with mutations and genotype calling. One advantage of customized software is data masking, or the ability of the user to display the genotype for mutations determined to be appropriate, such as only those mutations associated with the diseases for which testing has been requested by the ordering physician.

Quality control (QC) and controls. It may not be feasible to include genomic DNA (gDNA) for each positive assay control in each run due to reagent cost and batch size limitations. QC on a new lot of beads should include gDNA-based testing for each mutation. However, at a minimum, during routine test-

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ing, it is recommended that each run include at least one positive assay control and that all positive controls be tested on a rotating basis. The use of either genomic or synthetic compound heterozygotes can also maximize the number of positive controls while limiting the number of reaction wells used. The last sample in each batch should be a no-template control, to assess for reagent contamination by previous or current amplicons. The ratio of wild type to mutant signal, adjusted for background for each control, should fall into previously set ranges that maximize the signal to noise ratio and the no-template controls should fall below an arbitrary preset detection limit.

Amplification refractory mutation system (ARMS)

Overview. ARMS is based on the observation that oligonucleotide primers that are complementary to a given DNA sequence except for a mismatch (typically at the 3′ OH residue) will not, under appropriate conditions, function as primers in a PCR reaction. For genotyping, paired PCRs are performed for each mutation tested. One primer (common primer) is used in both reactions, whereas the other is either specific for the mutant or wild-type sequence. In principle, ARMS tests can be developed for any single base pair change or small deletions/insertions. Achieving acceptable specificity is dependent on primer selection and concentration. Use of longer primers (e.g., 30 vs. 20 bp) and inclusion of control reactions have been reported to improve specificity. Primers and conditions for multiplex reactions must be selected so that the relative yields of PCR products are balanced and the PCR products can be adequately resolved with gel electrophoresis.

Laboratory developed primer sets must be validated to ensure desired performance characteristics, and new reagent lots should be compared to a previous lot to ensure consistency in performance and robustness. One commercial set of ASRs for ARMS is available to detect mutations associated with the following diseases: TSD, FD, CD, FAC, MLIV, NPD-A, glycogen storage disease type 1a (Von Gierke disease), Bloom syndrome and GD. The detection of 20 mutations associated with these diseases can be accomplished using three separate sets of ASRs, with each containing reagents for one multiplex reaction to detect mutations in 1 to 4 genes. Although the manufacturer performs a level of performance evaluation on these reagents, the laboratory must also complete an internal validation to assess proficiency before use on patient samples.

Controls. Internal control reactions are not required if mutant and wild-type ARMS reactions are combined in the same test. However, for screening purposes, multiplexing mutant ARMS reactions without paired wild-type reactions can result in significant cost savings. Internal controls (additional control primers that amplify unrelated sequences) can be included in each multiplex reaction to ensure that DNA samples will generate at least one PCR product in each tube and reduce the likelihood of false negative results. Negative and positive control samples must be run with each assay but the laboratory may determine that it is not feasible to include all mutation controls in each run due to batch size limitations. Pooled positive DNA control samples can be utilized to allow efficient inclusion of the most common mutation controls in each run. Remaining positive controls can be tested on a rotating basis.

Visualization and interpretation of results. PCR products are separated by gel electrophoresis and visualized by ethidium bromide staining (or other DNA specific stain) and UV transillumination. Individual test results are interpreted by review of the banding pattern in comparison with a molecular weight standard. The disadvantage of assays without paired wild-type reactions is that they do not discriminate between the heterozygous and homozygous mutant state. Therefore, additional testing by another method must be performed to accurately interpret the results. Advantages of the ARMS method are that it is rapid (results can be obtained in one working day), reportedly reliable, and does not require expensive instrumentation.

Testing for diseases on an individual basis

For some multiplex methods (including the commercially available ARMS product) it might not be possible to limit testing to specific diseases and/or mutations. In this case, testing for some diseases might be performed as part of a multiplex assay even though testing for those particular diseases was not requested by the ordering physician or consented by the patient. State laws vary with respect to the duty of the laboratory to fully disclose all test results, even when a specific test was not ordered. Moreover there may be CLIA implications for reporting, and liability implications for not reporting, such results. Given both the clinical and legal uncertainties in this area, the ACMG recommends that each institution consult with their legal counsel for guidance on the best practice laboratory policy that best meets applicable requirements.

In the absence of clear guidance, in addition to offering a multiplex test, laboratories may wish to either offer testing for each disease individually or send specimens for single gene testing to another laboratory. Sometimes testing for individual diseases may be specifically requested by the ordering physician. Testing for specific mutations may be requested in certain circumstances, such as for a known familial mutation or prenatal testing when both parents are known to carry a specific mutation(s). Testing for individual mutations is also used when a mutation is identified using an ARMS-based assay without paired wild-type reactions to differentiate between the heterozygous and homozygous mutant state. Methods to detect common mutations in CD,[64] TSD,[65] and FD[16,50] using PCR and restriction enzymes have been published and are summarized in Table 7. Alternatively, other laboratory-developed methods can be used, if adequately validated. Primers can be developed using any commercially available primer design software package that helps to select optimum sets of primers based on Tm and salt concentration. In addition, software packages can assist in the identification of restriction enzymes sensitive to specific sequence changes.

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Table 7

Examples of restriction enzymes that can be used to detect common mutations in Canavan disease, Tay-Sachs disease, and familial dysautonomia

Disease	Mutation	Restriction enzyme	Effect of mutation on PCR amplicon
Canavan disease[64]	Y231X (693C→A)	*Mse*I	Creates enzyme recognition site
	E285A (854A→C)	*Eag*I or *Not*I	Creates enzyme recognition site
Tay-Sachs disease[65]	G269S	*Bst*NI	Destroys enzyme recognition site
	1278insTATC	*Hae*III	Insertion mutation increases size of restriction fragment
	1421 + 1G→C (IVS12)	*Dde*I	Creates enzyme recognition site
Familial dysautonomia[16,50]	2507 + 6T→C (IVS20)	*Cfo*I	Creates enzyme recognition site

The DNA fragments generated by restriction enzyme digestion are resolved using gel or capillary electrophoresis. It is recommended that the DNA fragments generated by PCR contain a recognition sequence for the enzyme, independent of the restriction site related to the presence or absence of the mutation. This constant site serves to ensure that the restriction enzyme is working properly. When possible, it is preferable to design restriction enzyme assays such that the mutation introduces a restriction enzyme recognition sequence rather than eliminating a site. Control samples with a known genotype corresponding to each class (homozygous wild-type, heterozygous and, when available, homozygous mutant) as well as no-DNA controls should be included for each assay.

Incorrect assignment of homozygosity

Sequence changes in primer binding sites or restriction enzyme recognition sequences may lead to a false positive homozygous mutant genotype or false negative wild-type genotype. With the exception of GD, an incorrect assignment of homozygous mutant is suggested when the indication is carrier screening. Parental testing or testing by an alternative method is recommended when the genotype does not correlate with reported phenotype or indication for testing. A polymorphism at the site of the CD Y231X mutation has been reported which may cause misinterpretation of molecular assays.[66,67]

Quality assurance

Laboratories should follow molecular pathology guidelines established by the College of American Pathology (CAP), be in compliance with the NIH-DOE Task Force on Genetic Testing,[68] and follow the ACMG Standards and Guidelines for Clinical Genetics Laboratories.[55] CAP offers a proficiency testing program for CD, FD, TSD, and CF. All aspects of testing, including pre- and postanalytical, must be in full compliance with regards to appropriateness of test ordering, interpretation, reporting, and counseling. Regardless of whether a laboratory-developed method or ASRs are used, laboratories must validate their assays, as well as state the analytical and clinical sensitivity and specificity according to the ACMG guidelines.[55]

Laboratory result interpretations (postanalytical)

When performing testing for multiple diseases, the laboratory may wish to consider consolidating the results for all of the diseases tested into one report, allowing easy review for the ordering health care provider. In addition to the items described in the current ACMG Standards and Guidelines,[55] the following elements should be included in the report: ethnicity, indication for testing, disease(s) and mutations tested, test method, test result, and the patient's residual carrier risk when negative results are obtained.

The patient's results

Model reports are included in the Appendix. The report should include a clear interpretation of the patient result as homozygous for a mutation (predicted affected), a compound heterozygote (predicted affected), heterozygous carrier (interpretation depends on whether the indication is carrier screening or diagnostic testing) or negative (interpretation depends on whether the indication is carrier testing with or without a family history or diagnostic). In cases where mutations are identified, the disease associated with the presence of the mutation and the name of specific mutation(s) should be included.

All positive results for carrier screening or diagnostic testing should state that 1) genetic counseling is recommended and 2) testing is available for at-risk family members. In addition, when sequential preconception or prenatal carrier testing is done, a positive result on one partner should include the recommendation of testing the other partner for the relevant disease. All individuals who have a positive family history should be offered the opportunity to receive formal genetic counseling.

Comments on individual residual risk and reproductive risk for couples (when appropriate) should be included in the patient report or provided to the referring health care professional. Comments should be written to be consistent with current HIPAA guidelines. Table 8 is only intended to be used for persons of full Ashkenazi Jewish ancestry. Previously published carrier frequencies and sensitivities of the minimum mutation panel were used in Bayesian calculations to generate this table. This table is intended for use in screening of reproductive couples who have no family history of TSD, CD, or FD. For individuals with a positive family history, the calculations would be different and would be based upon pedigree information. It is the laboratory's responsibility to provide this type of information, specific for the population it serves.

Recommendations for testing partner when a carrier is identified through sequential screening

When one partner is identified as a carrier, then testing for that same disease should be offered to their partner. If the partner is of full Ashkenazi Jewish descent, then the couple's

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Table 8
Summary of residual risks that Ashkenazi Jewish partners carry a mutation in the same gene and the risk for an affected child, based on molecular test results[a]

Disease (mutations tested)	Carrier rate	Mutations detected	Testing performed/results			Approximate residual risk (1 in N)[b]		
			None	Negative	Positive	Individual[c]	Couple[d]	Fetus[e]
Tay-Sachs disease (1278insTATC, 1421 + 1G→C and G269S)	1 in 31	98.9%	XX[a]			31	960	3,800
				XX		2,800	>1,000,000	>1,000,000
					XX	1	1	4
			X	X		—	87,000	350,000
			X		X	—	31	120
				X	X	—	2,800	11,000
Canavan disease (E285A and Y231X)	1 in 41	97.4%	XX			41	6,700	27,000
				XX		1,540	>1,000,000	>1,000,000
					XX	1	1	4
			X	X		—	63,000	253,000
			X		X	—	41	160
				X	X	—	1,540	6,200
Familial dysautonomia (2507 + 6T→C and R696P)	1 in 31	99.4%	XX			31	960	3,800
				XX		5,000	>1,000,000	>1,000,000
					XX	1	1	4
			X	X		—	155,000	620,000
			X		X	—	31	120
				X	X	—	5,000	20,000

[a]These calculations pertain only to individuals of full Ashkenazi Jewish ancestry. Determining the residual risks for individuals who are not exclusively Jewish requires additional Bayesian calculations.
[b]An "X" indicates the result for one of the partners.
[c]A partner's carrier risk, based on the test results.
[d]The product of each partner's carrier risk.
[e]The couples' carrier risk times ¼.

risk of having an affected child can be calculated, based on the test results. If the partner is non-Jewish, it may be difficult to perform an accurate risk assessment unless the carrier frequency and mutation detection rate is known for that individual's ethnic origin. For TSD, carrier screening using the HEX A enzyme assay should be offered to a non-Jewish partner of a known carrier, DNA testing is not recommended for non-Jewish individuals.

SUMMARY OF RELATED POLICY STATEMENTS

The American College of Obstetricians and Gynecologists (ACOG)

Screening for Canavan disease[69]

Recommends preconception or prenatal carrier screening for couples of Ashkenazi Jewish ancestry. If only one partner is of high risk (Jewish or positive family history) then that partner should be tested first, with testing offered to the partner if the result is positive. Prenatal diagnosis by chorionic villus sampling (CVS) or amniocentesis is recommended when both partners are known carriers of CD.

Prenatal and preconception carrier screening for genetic diseases in individuals of eastern European Jewish descent[5]

Recommends determining whether the pregnant patients or individuals considering pregnancy are at high risk (Ashkenazi Jewish or positive family history of TS, CD, CF, FD, FA-C, NPD-A, MLIV, Bloom syndrome, or GD). Preconception or prenatal carrier screening for TSD, CD, CF and FD should be offered to individuals of Ashkenazi Jewish ancestry and individuals with a positive family history. If patients inquire about the availability of testing for other conditions (MLIV, NPD-A, FA-C, Bloom syndrome, and GD), educational material can be made available so that patients can make an informed decision regarding testing. When only one member of a couple is Jewish, that partner should be screened first. The other partner should be offered screening if the high risk partner is a carrier. Genetic counseling is recommended and prenatal testing should be offered when both members of the couple are carriers of the same disorder. In addition, mutation carriers should be encouraged to share their carrier status with their at-risk relatives and the availability of carrier screening.

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Screening for Tay-Sachs disease[37]

Recommends preconception screening for TSD if both members of a couple are at high risk (Ashkenazi Jewish, French Canadian, or positive family history). When one member of a couple is high risk, but the other partner is not, the high risk partner should be screened first. Testing should be offered to the other partner if the high risk partner is found to be a carrier. Screening can be performed by DNA testing or biochemical screening; however, biochemical screening is recommended for non-Jewish individuals or individuals of mixed ethnicity. When biochemical screening is performed in pregnant women or women taking oral contraceptives, leukocyte testing must be used. Ambiguous biochemical screening tests results or positive screening test results should be followed up by DNA testing to confirm the presence of either pathogenic mutations or pseudodeficiency alleles. Prenatal diagnosis should be offered if both partners are determined to be carriers of TSD or if one partner is a carrier and the other has an indeterminate or inconclusive carrier status that cannot be resolved.

Update on carrier screening for Cystic Fibrosis[70]

Recommends that information about CF screening be made available to all couples. It is reasonable to offer CF screening to all couples regardless of race or ethnicity. However, either sequential or concurrent preconception/prenatal screening should be offered to all Caucasian, European, or Ashkenazi Jewish couples. Extended CF mutation panels beyond the 23 currently recommend mutations is not recommended for routine screening, but may be considered for diagnostic testing, individuals with a family history of CF, males with CBAVD or after a positive newborn screen. Genetic counseling is recommended for all positive-positive couples and prenatal diagnosis should be offered. When two mutations are identified in an individual undergoing carrier screening in whom a diagnosis of CF has not been made, the patient should be referred to a specialist in CF for further evaluation.

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APPENDIX

Model laboratory reports

Reported ethnicity: Ashkenazi Jewish

Indication: screening/carrier test/negative family history

Methods: Mutation studies were performed by PCR-based DNA amplification using the ARMS. The presence of any mutation was confirmed by restriction fragment length polymorphism analysis or bi-directional DNA sequencing. The mutations examined are as follows: TSD (c.1278insTATC, G269S, and c.1421 + 1G>C in IVS12), CD (E285A and Y231X), and FD (R696P and 2507 + 6T>C in IVS20). This test was developed and its performance characteristics determined by this laboratory. It has not been cleared or approved by the FDA. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is regulated under CLIA of 1988.

Comment: Possible diagnostic errors include sample mix-ups and genotyping errors, which can result from trace contamination of PCRs, maternal cell contamination of fetal samples and from rare polymorphisms, which interfere with analysis. Mistaken paternity may be inadvertently identified. Mutation analysis should be combined with phenotypic and pedigree data for the most accurate interpretation.

Example of a negative report

Result: Negative for the mutations analyzed.

Interpretation: These results indicate that this individual is not a carrier of the mutations tested for in this laboratory. The remaining carrier risks for persons of full Ashkenazi Jewish heritage, as determined by Bayesian calculation, for the diseases tested are as follows:

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Table A

Disease	Mutations detected (%)	Estimated carrier risk	
		Before test	After negative test
Canavan disease	97.4	1 in 41	1 in 1540
Familial dysautonomia	99.4	1 in 31	1 in 5000
Tay-Sachs disease	98.9	1 in 31	1 in 2800

Example of a positive report

Result: Heterozygous for the 1278insTATC TSD mutation. No other mutation tested for in this assay was identified.

Interpretation: One copy of the 1278insTATC TSD mutation was identified, indicating that this individual is a carrier for TSD. Carrier testing for TSD is recommended for this individual's reproductive partner and at-risk relatives. Genetic counseling is recommended.

The remaining carrier risks for persons of full Ashkenazi Jewish heritage for the other diseases tested, as determined by Bayesian calculation, are as follows:

Table B

Disease	Mutations detected (%)	Estimated carrier risk	
		Before test	After negative test
Canavan disease	97.4	1 in 41	1 in 1540
Familial dysautonomia	99.4	1 in 31	1 in 5000

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