Chapter 15

Neurodegenerative Disorders

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HUNTINGTON DISEASE

Molecular Basis of Disease

Huntington disease (HD) is an autosomal dominant neurodegenerative disorder clinically characterized by the presence of choreiform movements, psychiatric sequelae, and dementia. While the majority (>90%) of HD patients become clinically symptomatic in adulthood, 5% to 10% of patients present with the juvenile-onset form of the disease, which is almost invariably associated with inheritance of the mutant allele from a symptomatic father. Unlike the adult-onset form of the disease, juvenile HD is generally characterized by the presence of progressive rigidity, seizures, ataxia, and dystonia.

The HD gene (*IT15*) is located on chromosome 4p16. The gene spans more than 200 kilobases (kb) and contains 67 exons. The encoded protein, huntingtin, is a 3,136 amino acid protein with an approximate molecular weight of 350 kilodaltons (kDa) that shares no known homologies with other characterized proteins. HD is caused by a toxic gain-of-function mechanism associated with the expansion of a polyglutamine tract within the protein that is translated from a CAG repeat region within exon 1 of the gene. The CAG repeat length is highly polymorphic in the general population,[1] with the largest normal allele currently defined as carrying ≤26 CAG repeats. Alleles carrying ≥40 CAG repeats, identified in symptomatic patients with a presumptive clinical diagnosis of HD, are considered diagnostic. Alleles with 27 to 35 CAG repeats are defined as "mutable normal or intermediate alleles." While alleles in this size range have yet to be convincingly associated with an HD phenotype, they can be meiotically unstable in sperm and result in the expansion of paternally derived alleles.[2] Alleles found to carry 36 to 39 CAG repeats are defined as "HD alleles with reduced penetrance." Alleles in this size range have been found in both clinically as well as neuropathologically confirmed HD patients and in elderly asymptomatic individuals.[2]

Clinical Utility of Testing

The direct quantitative analysis of HD CAG repeat number is clinically available in a large number of clinical molecular laboratories.[3] As HD appears to be a genetically homogeneous disease, molecular testing has been routinely utilized with confidence for diagnostic, predictive, and prenatal purposes. In the case of predictive testing, professional standards strongly encourage that this test be offered only to individuals who have reached the statutory age of majority (generally 18 years of age). For adults, a formal multidisciplinary predictive testing protocol is offered at many sites for those desiring determination of their carrier status before the onset of symptoms. The recommended presymptomatic protocol includes pretest counseling and evaluation of the individual being tested by a clinical geneticist, genetic counselor, psychologist or psychiatrist, and neurologist over several visits, with the option to not receive test results even after testing has been completed. With few exceptions, interpretation of laboratory results is unambiguous and the resulting genetic counseling consultations follow those for other autosomal dominant adult-onset disorders.

Available Assays

Detection of HD CAG repeat expansions can be determined by both PCR and Southern blot methods. For PCR, several sets of primers, assay conditions, amplicon separation, and detection techniques have been published.[2] Regardless of the particular PCR test method employed, optimization of the assay conditions and post-PCR analyses are essential to ensure accurate and unambiguous quantitation of the HD CAG repeat length (Figure 15-1). CAG sizing anomalies have been observed in both comparative studies of ^{32}P incorporation methods[2] and comparative post-PCR analyses utilizing agarose, capillary, and denaturing polyacrylamide gel electrophoretic methods.

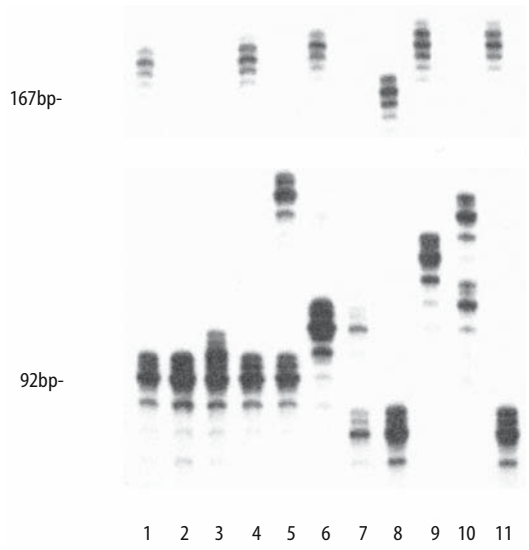

Figure 15-1. PCR genotyping of the Huntington disease polymorphic CAG repeat utilizing ^{32}P-dCTP incorporation and the AI/C2 primer pair.[4,5] Separation and sizing of alleles was performed on a 35 cm × 43 cm 6% denaturing polyacrylamide gel followed by autoradiography. The numbers of CAG repeats in each of the two alleles are 17/44, 17/17, 17/18, 17/44, 17/25, 19/45, 15/19, 15/40, 22/45, 20/24, and 15/45 for patients 1 to 11, respectively.

While PCR testing has a sensitivity of approximately 99%, the fidelity of amplification of very large alleles is not empirically known. However, conventional PCR protocols reproducibly amplify alleles in the 115 to 120 CAG repeat range,[2] and modified PCR protocols (long-template PCR) have enabled detection of an approximately 265 CAG repeat allele in a patient with juvenile-onset disease.[6] As such, accurate quantitation of patient amplicon sizes should be empirically determined by comparison to appropriate external or internal standards. These generally include M13 sequencing ladders, cloned reference standards, and appropriate normal and abnormal patient controls with independently verified repeat sizes, usually by sequence analysis of the CAG repeat.

As the length of the polymorphic CAG repeat alone is associated with HD, patient genotyping based on the use of a single primer pair that amplifies both the CAG and adjacent CCG repeat[2] should be discouraged. The CCG repeat, which lies 12 base pairs (bp) 3′ of the HD CAG repeat, has been shown to be polymorphic and as a result may lead to diagnostic inaccuracies for both normal and HD allele sizing. Furthermore, polymorphisms surrounding or within the CAG tract have been identified with a collective frequency of approximately 1%.[2] These nucleotide substitutions can be categorized into two groups: (1) those that modify primer-annealing sites and (2) those that result in the loss of sequence interruption between the CAG and CCG tracts. With the first category, nucleotide changes may result in the misinterpretation of genotyping data due to an allele-specific amplification failure associated with improper primer annealing. In the second category, rare A-to-G substitutions within the intervening 12 bp segment between the CAG and CCG tracts can result in increased meiotic instability of the tract as well as miscalculation of uninterrupted CAG repeat length based on conventional calculation formulas.[2]

Southern blot methods, although not generally used for routine diagnostic testing, are often essential for the identification or confirmation of very large expansions typically associated with juvenile-onset HD, because these alleles are typically refractory to robust amplification by conventional PCR. Furthermore, this method may be useful for the confirmation of patients (generally children) who appear to be homozygous for two normal-sized alleles.[2]

Interpretation of Test Results

PCR testing for the HD CAG repeat has a reported sensitivity of 99%. The remaining 1% of patients represent HD phenocopies, and to date at least two distinct genetic loci (*HDL1* and *HDL2*) have been identified.[2] Testing specificity is 100%.[1] In summary, the interpretative challenges in HD testing are primarily related to the need to recognize that CAG ranges and descriptors may be modified over time. For reporting of HD results, the American College of Medical Genetics (ACMG) has recently established updated guidelines regarding the definitions of CAG repeat range descriptors and interpretative guidelines for genetic counseling purposes.[2]

Laboratory Issues

There is currently no commercially available in vitro diagnostic test kit for HD. The College of American Pathologists (CAP) offers HD proficiency challenges twice a year as part of the CAP/ACMG Molecular Genetics Laboratory (MGL) Survey. Genotyped HD patient DNA for use as controls can be obtained from the Coriell Cell Repositories (Camden, NJ; http://ccr.coriell.org/).

AUTOSOMAL DOMINANT CEREBELLAR ATAXIAS AND FRIEDREICH ATAXIA

Molecular Basis of Disease

The autosomal dominant cerebellar ataxias (ADCAs) collectively represent a clinically heterogeneous group of neurological disorders. To date, at least 27 distinct loci have been identified, and clinical testing is presently available for 12 of these (Table 15-1). Because the collective incidence of these disorders is high in both unselected and selected ataxia cohorts[7,8] and the degree of clinical overlap among the various ADCAs is extensive, molecular genetic testing is a valuable diagnostic tool. Most of the molecularly characterized ADCAs (dentatorubro pallidoluysian atrophy or DRPLA and SCA1, 2, 3, 6, 7, 8, 10, 12, and 17) are

Table 15-1. Molecular Classification of the Autosomal Dominant Cerebellar Ataxias (ADCA) and Friedreich Ataxia (FRDA)

Locus	Inheritance	Chromosome	Gene Product	Clinical Test[1]
DRPLA	AD	12p13	Atrophin-1	Yes
SCA-1	AD	6p23	Ataxin-1	Yes
SCA-2	AD	12q24	Ataxin-2	Yes
SCA-3	AD	14q24	Ataxin-3	Yes
SCA-4	AD	16q22	Puratrophin-1	No
SCA-5	AD	11p11	Spectrin beta chain, brain 2	Yes
SCA-6	AD	19p13	Voltage-dependent P/Q-type calcium channel a-1A subunit	Yes
SCA-7	AD	3p21	Ataxin-7	Yes
SCA-8	AD	13q21	Not named	Yes
SCA-9 (not assigned)	AD			
SCA-10	AD	22q13	Ataxin-10	Yes
SCA-11	AD	15q14	Unknown	No
SCA-12	AD	5q31	Protein phospatase 2, regulatory subunit B	Yes
SCA-13	AD	19q13	Potassium voltage-gated channel subfamily 3, mumber 3	No
SCA-14	AD	19q13	Protein kinase C, gamma type	Yes
SCA-15	AD	3p26	Unknown	No
SCA-16	AD	8q22	Unknown	No
SCA-17	AD	6q27	TATA-box binding protein	Yes
SCA-18	AD	7q22	Unknown	No
SCA-19	AD	1q21	Unknown	No
SCA-20	AD	11cen	Unknown	No
SCA-21	AD	7p21	Unknown	No
SCA-22	AD	1p21	Unknown	No
SCA-23	AD	20p13	Unknown	No
SCA-25	AD	2p21	Unknown	No
SCA-26	AD	19p13	Unknown	
SCA-27	AD	13p34	Fibroblast growth factor 14	No
SCA-28	AD	18p11	Unknown	No
FRDA	AR	9p13	Frataxin	Yes

Sources: See GeneTests (www.genetests.org), The Human Gene Nomenclature Database (www.gene.ucl.ac.uk/nomenclature/), and The Neuromuscular Disease Center, Washington University, St Louis, MO (www.neuro.wustl.edu/neuromuscular/index.html).
[1]DNA-based testing.
AD, autosomal dominant; AR; autosomal recessive.

associated with reiterated repeat expansion mutations. DRPLA and SCA1, 2, 3, 6, 7, 12, and 17 are associated with CAG trinucleotide repeat expansion mutations. SCA8 is associated with a CTG expansion mutation, and SCA10 with an ATTCT pentanucleotide repeat expansion.

In the majority of these disorders the location of the repeat is exonic (DRPLA and SCA1, 2, 3, 6, 7, and 17), and the mechanism of disease pathogenesis (with the exception of SCA6) is thought to involve a toxic gain of function in the protein. With some exceptions, SCA5 is associated with the spectrin beta chain,[3] the function of the normal gene product is not known. SCA6 is associated with a CAG expansion in the *CACNA1A* gene, which encodes the voltage-dependent P/Q-type calcium channel α-1A subunit. SCA12 is associated with a CAG expansion in the 5′ UTR of the *PPP2R2B* gene, which encodes a brain-specific regulatory subunit of the protein phosphatase PP2A.[9] SCA13 with the protein voltage-gated channel subfamily C member 3,[3] SCA14 with the protein kinase C, gamma type,[3] SCA17 is associated with a CAG repeat expansion in the TATA-binding protein (*TBP*) gene, which encodes a general transcription initiation factor.[10] SCA8 is associated with a CTG expansion within the 3′ untranslated exon of a fully processed transcript of a gene of unknown function[11] and SCA10 is associated with an intronic expansion of a pentanucleotide (ATTCT) sequence within the *SCA10* gene encoding a protein of unknown function.[12]

Friedreich ataxia (FRDA) is the most common form of hereditary ataxia, with a prevalence of approximately 2 to 4 per 100,000 and a carrier frequency of approximately 1 in 90. The disease has an autosomal recessive mode of inheritance. FRDA is clinically characterized by the onset of gait and limb ataxia before the age of 25 years, decreased deep tendon reflexes, dysarthria, pyramidal signs, Babinski responses, and decreased position or vibration sense (or both) in the lower limbs. Approximately 65% of patients also develop a cardiomyopathy, while approximately 10% of patients develop diabetes. Atypical clinical presentations have been reported in approximately 25% of patients and generally have included later ages of onset (after 25 years of age), retained reflexes, and slower disease progression. In addition, genetically confirmed patients with more complex phenotypes have recently been reported and include individuals with idiopathic spastic paraparesis, motor and sensory neuropathy, limb and axial dystonia, and chorea with myoclonus.[13]

FRDA is caused by mutations in the *FRDA* gene that encodes the protein frataxin. Frataxin is a 210 amino acid protein that localizes to the mitochondrial inner membrane, where it is required for mitochondrial iron

homeostasis. Approximately 96% of patients are homozygous for GAA expansion mutations within intron 1 of the *FRDA* gene. However, the remaining 4% of patients are compound heterozygotes for a GAA expansion on one allele and a point mutation on the other. Both mutation types, however, are gene inactivating.[13]

Clinical Utility of Testing

The utility of offering testing for the molecularly characterized ADCAs and FRDA is very high. For the ADCAs, diagnostic, predictive, as well as prenatal testing can be performed with confidence. Although allelic heterogeneity has been reported in FRDA (i.e., point mutations in compound heterozygotes), the vast majority of FRDA patients have homozygous expansions of the GAA repeat; hence, diagnostic, predictive, and prenatal testing can also be offered with confidence. As is the case with HD, predictive testing for the ADCAs should be offered only to individuals who have reached the statutory age of majority (generally 18 years age). For adults, a formal multidisciplinary predictive testing protocol, similar to the one utilized for HD predictive testing, should be offered to those desiring determination of their carrier status. Again, with few exceptions, interpretation of the laboratory result is unambiguous, and the resulting genetic counseling consultations follow those for other autosomal dominant adult-onset disorders. For FRDA families, appropriate counseling consistent with an autosomal recessive pattern of inheritance is required.

Available Assays

Detection and quantitation of the repeat expansions associated with the ADCAs can be determined by both PCR and Southern blot methods, and the choice of methodology is dependent on the characteristic size range for the disease-specific expansion mutation. For example, very large expansions, such as those routinely seen in SCA10, require the use of Southern blot, whereas those seen in other ADCAs (SCA6, for example) are amenable to detection by routine PCR analysis. There are some notable exceptions, however, which are described below. As already alluded to for HD, regardless of the particular PCR method employed, assay conditions and post-PCR analyses should be optimized to ensure accurate and unambiguous quantitation of repeat length. Furthermore, CAG sizing anomalies for ADCA testing also have been observed in comparative studies of capillary and denaturing polyacrylamide gel electrophoretic methods.[14] As such, accurate quantitation of patient amplicon sizes should be empirically determined by comparison to appropriate external or internal standards.

One of the important methodological considerations for quantitation of expansion mutations in general is the recognition of the existence of "extreme expansions" associated with several of the SCAs. For example, both the infantile and juvenile-onset forms of SCA2 and SCA7 have been associated with large, or "extreme," expansions generally greater than 200 CAG repeats.[15] In a situation that is analogous to the large expansions of juvenile-onset HD, these alleles can be refractory to efficient PCR or difficult to separate using conventional polyacrylamide gel electrophoresis (PAGE). Test results that are apparently "homozygous normal" genotypes in infants or children with a high index of clinical suspicion of SCA2 or SCA7 should be confirmed using a Southern blot test that can detect large expansions (Figure 15-2a and b).[17] This testing strategy also is important for SCA8, since repeat lengths of greater than 800 have been described in adults with SCA8.[18] Detailed current information regarding repeat ranges, clinical descriptors, and disease-specific commentaries for the ADCAs is available on the web.[19]

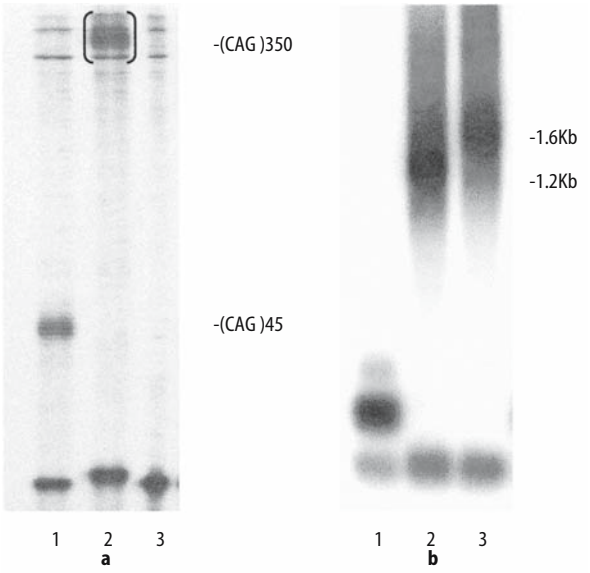

Figure 15-2. Molecular testing for the SCA2 CAG repeat. (a) PCR genotyping of the SCA2 polymorphic CAG repeat utilizing the UH13/UH10 primer pair[16] is shown for two related patients (lane 1, mother; lane 2, son) with adult-onset and juvenile-onset SCA2, respectively.[15] Separation and sizing of alleles was performed on a 35 cm × 43 cm 6% denaturing polyacrylamide gel followed by autoradiography. The numbers of CAG repeats in each of the two alleles are 22/45 (lane 1, affected mother run in duplicate), 23/~350 (lane 2, affected child run in duplicate), and 22/22 (lane 3, normal unaffected control). (b) PCR-Southern blot analysis[17] of the same mother-child samples shown in Figure 15-2a, confirming the presence of the extreme expansion in the affected son. Allele sizes are 22/45 (lane 1, affected mother), 23/~350 (lane 2, affected child), and 23/~400 (lane 3, positive extreme expansion control). (Figure 15-2b courtesy of Dr. Karen Snow-Bailey, Auckland District Health Board, Auckland, New Zealand.)

Analysis of the FRDA GAA expansion mutation is generally performed by long-template PCR (Figure 15-3a and b) or by Southern blot analysis. Although the fidelity of this assay is high, PCR artifacts resembling expansions can be seen, particularly in samples with two normal-sized alleles (Figure 15-3a, lane 4). The expansion artifacts are thought to be heteroduplexes, as they do not appear when amplicons are analyzed under denaturing conditions. These artifacts should not result in significant diagnostic errors for patients who are clearly heterozygous for two normal-sized

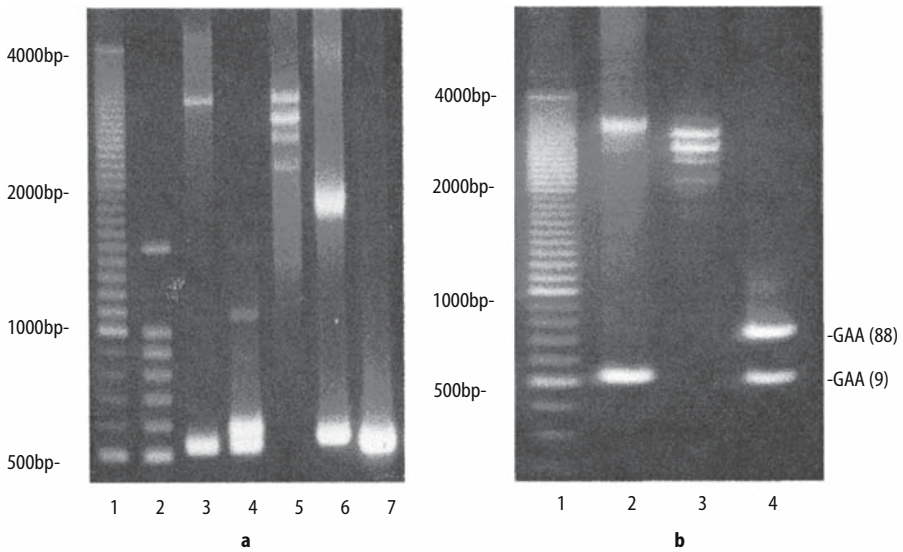

Figure 15-3. PCR analysis of the FRDA polymorphic GAA expansion mutation utilizing the 104F/629R primer pair[20] and the Expand Long Template PCR System (Roche Diagnostics, Mannheim, Germany). (a) Lane 1, 100 bp step ladder G6951 (Promega, Madison, WI); lane 2, 100 bp ladder G2101 (Promega); lane 3, FRDA carrier with ~850 GAA repeats; lane 4, normal individual with allele sizes of 9 and 26 GAA repeats; lane 5, FRDA patient with allele sizes of ~800/~900 GAA repeats; lane 6, FRDA carrier with ~450 GAA repeats; lane 7, normal control. (b) Lane 1, 100 bp step ladder G6951 (Promega); lane 2, FRDA compound heterozygote (see Figure 15-4) with ~1000 GAA repeats; lane 3, FRDA patient with allele sizes of ~800/~900 GAA repeats; lane 4, FRDA carrier with allele sizes of 9 and 88 GAA repeats (confirmed by DNA sequencing). PCR products were separated through 1% agarose on a 10.5 cm × 14 cm horizontal gel and the alleles visualized after ethidium bromide staining.

alleles, as the intensity of the true alleles is usually much greater than that of the heteroduplexes. Ambiguities may remain, however, because the resolving power of 1% agarose gels for amplicons in the 500 bp range is limited. These can be clarified, if necessary, by either Southern blot or denaturing PAGE.

Interpretation of Test Results

PCR tests for the ADCAs have a sensitivity of >99%, and complete testing including Southern blot has a sensitivity of close to 100%. For the reporting of results, interpretation of the findings requires the integration of available clinical information, repeat size, and its clinical descriptor (i.e., normal, intermediate, or abnormal). Again, the subtleties of effective genetic counseling for the ADCAs require an understanding of the significance of repeat sizes and an appreciation that repeat ranges and descriptors may change over time.[19] PCR tests for FRDA have a sensitivity of approximately 96%, but the presence of a frataxin point mutation must be considered in patients with a high degree of clinical suspicion and only one expanded GAA allele (Figure 15-3b, lane 2). In such situations, referral to a laboratory that offers point mutation analysis is strongly recommended. See also Figure 15-4.

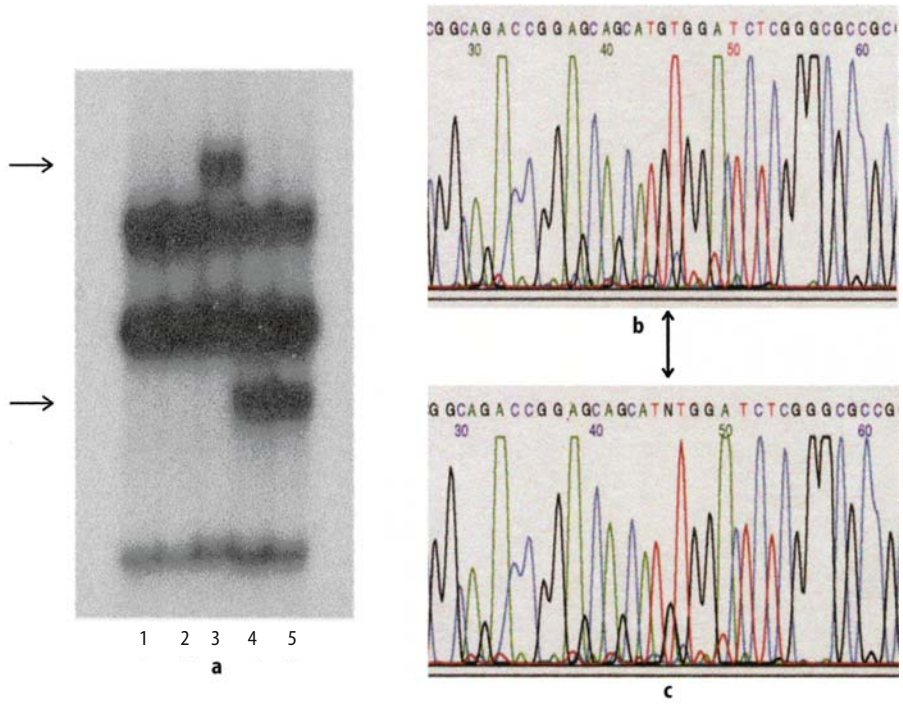

Figure 15-4. (a) SSCP analysis of the *FRDA* gene exon 1 amplicon revealing the presence of abnormal conformers (→) in two FRDA compound heterozygotes. Lanes 1 and 2, exon 1 amplicon from a normal control; lane 3, FRDA compound heterozygote carrying a G→A transition at nucleotide 3 of the *FRDA* gene;[21] lanes 4 and 5 (duplicate), FRDA compound heterozygote carrying an A→C transversion at nucleotide 1 of the *FRDA* gene. (b, c) Representative exon 1 electropherograms from a normal individual (b) and the 3G→A/GAA_{1000} FRDA compound heterozygote (c) shown in Figure 15-4a, lane 3, confirming the presence of the mutation in the heterozygous state (↕).

Laboratory Issues

There are currently no in vitro diagnostic test kits commercially available for the ADCAs or FRDA. The CAP offers proficiency testing twice a year as part of the CAP/ACMG MGL Survey that includes challenges for SCA1, 2, 3, 6, and 7 as well as FRDA.

ALZHEIMER DISEASE

Molecular Basis of Disease

Alzheimer disease is an adult-onset slowly progressive dementia with gradual erosion of intellectual function. Alzheimer disease is the most common form of dementia in the elderly, accounting for approximately 50% of the dementia in patients over age 85 years. Alzheimer disease can be categorized as either sporadic (75% of cases) or familial (25%). The 25% of familial cases are associated with either late- (20%) or early-onset disease (5%) with autosomal dominant/multifactorial or classic autosomal dominant inheritance patterns (Table 15-2). Of the five identified Alzheimer disease loci, four have been characterized at the gene and protein levels (AD1-4) and clinical (DNA) testing is available for three (AD2-4).

Late-onset Alzheimer disease (AD2), representing approximately 20% of the familial cases, has been associated with the presence of the apolipoprotein E (APOE) ε4 allele in many large studies.[22] APOE is a plasma protein involved in the transport of cholesterol and is a component of the very low-density lipoprotein (VLDL). The protein exists in three isoforms (apoE2, E3, and E4) encoded for by three alleles (ε2, ε3, and ε4). In the general population, the ε3 allele is the most common allele, found in approximately 77% of individuals. As stated, while many studies have corroborated the observation that the presence of an ε4 allele in an individual with dementia increases the probability that the patient has Alzheimer disease, this association is not complete. In patients with a clinical diagnosis of Alzheimer disease, the presence of an ε4/ε4 genotype is virtually diagnostic (sensitivity of approximately 97%); however, since approximately 45% of Alzheimer disease patients do not have an ε4 allele, APOE genotyping is not specific for Alzheimer disease.[22,23]

In contrast to late-onset Alzheimer disease, pathologic mutations in at least three genes have been associated with the development of early-onset familial Alzheimer disease (EOFAD). AD1, comprising approximately 10% to 15% of EOFAD, is associated with mutations in the gene encoding the amyloid precursor protein (APP), a 110 to 130 kDa ubiquitously expressed protein. The small proteolytic fragment of APP, $A\beta_{1\text{-}42}$, is found as a major component of amyloid plaques, the neuropathological hallmark of Alzheimer disease. Over a dozen APP missense mutations have been described, with one mutational hotspot (V717) described in multiple kindreds. AD3 (accounting for as much as 70% of EOFAD) and AD4 (accounting for less than 5% of EOFAD) are both associated with mutations in two highly homologous proteins, presenilin 1 and presenilin 2 (PSEN1 and PSEN2). The specific functions of these proteins are not completely understood. Expression of both PSEN1 and PSEN2 mutations in transfected cells has demonstrated an upregulatory effect on $A\beta_{1\text{-}42}$ secretion, suggesting a role in the processing and secretion of APP and its amyloidogenic peptide. To date, more than 40 missense mutations have been identified in AD3 kindreds, whereas only three mutations have been reported in AD4 families.[22,23]

Table 15-2. Molecular Classification of Alzheimer Disease (AD)

Locus	Inheritance	Chromosome	Gene Product	Clinical Test[1]
AD1	AD	21q21.3	APP[2]	Yes
AD2	AD/Multifactorial[3]	19q13	APOE[4]	Yes
AD3	AD	14q24	PSEN1[5]	Yes
AD4	AD	1q31	PSEN2[6]	Yes
AD5	AD/Multifactorial	12q11	Unknown	No

Source: See GeneTests (www.genetests.org).
[1] DNA-based testing.
[2] APP (amyloid precursor protein).
[3] risk-factor/genetic susceptibility.
[4] APOE (apolipoprotein E).
[5] PSEN1 (presenilin 1).
[6] PSEN2 (presenilin 2).
AD, autosomal dominant

Clinical Utility of Testing

Although APOE represents a risk factor for late-onset familial and sporadic AD, the presence of an ε4 allele is neither necessary nor sufficient for the development of Alzheimer disease. As such, the use of *APOE* genotyping in the clinical setting remains controversial. As a diagnostic adjunct in the clinical evaluation of a patient with late-onset dementia, the test may have some demonstrable clinical utility; however, there is general agreement that this test should not be used for predictive purposes and should never be considered for prenatal testing. In contrast, within the context of a high degree of clinical suspicion and the documentation of a family history of Alzheimer disease, DNA diagnostic testing for both AD3 and AD4 has demonstrable utility. Confirmation of a pathologic mutation in a proband will establish a diagnosis and clarify the EOFAD subtype such that both presymptomatic testing for appropriate family members and prenatal testing for at-risk pregnancies could be offered.

Available Assays

APOE genotyping can be performed by traditional polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis or by the Invader method (Third Wave Technologies, Madison, WI).[24] Detection of pathologic mutations in *APP*, *PSEN1* and *PSEN2* requires direct DNA sequencing.

Interpretation of Test Results

Interpretation of an *APOE* genotype requires the concurrent evaluation of other clinical information available at the time of testing and should never be interpreted in the absence of this information. As such, testing is limited to situations where the index of clinical suspicion for AD is quite high. In contrast, the identification of a pathologic *PSEN1* or *PSEN2* mutation in a symptomatic proband is diagnostic. Testing sensitivity for *PSEN1* is estimated to be between 30% and 60%; therefore, a negative result needs to be interpreted with caution. While both presymptomatic as well as prenatal testing are theoretically possible in mutation-positive kindreds, the possible existence of incomplete or reduced penetrance must be discussed during genetic counseling.[25]

Laboratory Issues

No commercial in vitro diagnostic test kits are currently available for Alzheimer disease testing, although Third Wave Technologies (Madison, WI) does offer *APOE* genotyping reagents. Several *APOE* genotyped cell lines from Alzheimer disease pedigrees can be obtained from the Coriell Cell Repositories (Camden, NJ; http://ccr.coriell.org/). Formal proficiency testing for Alzheimer disease is not currently available.

PARKINSON DISEASE

Molecular Basis of Disease

Parkinson disease (PD) is a chronic, progressive, idiopathic neurodegenerative disorder of late onset. Clinically, the disease is characterized by the development of rigidity, bradykinesia, resting tremor, and postural instability. Neuropathologic changes are notable for the presence of Lewy bodies and the selective degeneration of dopaminergic neurons in the pars compacta and substantia nigra. Although most patients present with a late-onset sporadic form of the disease, the identification of familial forms of PD has clearly established the role of genetic factors in disease etiology and pathogenesis. To date, at least 11 forms of PD can be distinguished on a genetic basis, and genes for seven of these (PARK1, PARK2, PARK5, PARK6, PARK7, PARK8, and PARK11) have been identified (Table 15-3).

PARK1 is associated with early-onset autosomal dominant PD. Despite an earlier age of onset, clinically PARK1 patients have typical dopa-responsive PD as well as classic neuropathologic findings at autopsy. Two missense mutations in the α-synuclein (*SCNA*) gene at 4q21 have been identified to date. The first, A53T, was found in 13 families of Italian-Greek descent[26] and the second, A30P, identified in a single German kindred.[27] Both mutations appear to be highly penetrant. α-synuclein is a 140-amino-acid protein predominantly expressed in neuronal tissues. Although the function of α-synuclein is not well characterized, this protein is one of the major protein constituents of the Lewy body. One attractive hypothesis proposes that the mutant forms of the protein have a propensity to oligomerize and form toxic neuronal aggregates[28] that contribute to the formation of insoluble fibrils through the disruption of the cellular ubiquitin-proteosome pathway and ultimately neuronal cell death via an apoptotic mechanism.

PARK2, or juvenile-onset autosomal recessive PD, is associated with mutations in the *PARKIN* gene that maps to chromosome 6q25. The gene consists of 12 exons and encodes a 465 amino acid protein. Functionally, parkin contains a ubiquitin-like domain in the N-terminus and two RING finger domains in the C-terminus. Similar to other proteins containing RING finger domains, parkin has been shown to have an E3 ubiquitin ligase activity. The association of parkin mutations with an autosomal recessive form of PD suggests that it is the loss of E3 ubiquitin ligase activity that directly contributes to the pattern of neurodegeneration seen in PD. Like PARK1, this mechanism is thought to involve disruption of the ubiquitin-proteosome system, resulting in the abnormal accumulation of substrate proteins. To date, more than 35 pathologic loss-of-function *PARKIN* mutations have been identified.

Table 15-3. Molecular Classification of Parkinson Disease (PD)

Locus	Inheritance	Chromosome	Gene Product	Clinical Test[1]
PARK1	AD	4q21	SNCA[2]	Yes
PARK2	AR	6q25	Parkin	Yes
PARK3	AD	2p13	Unknown	No
PARK4	AD	4p15	Unknown	No
PARK5	AD	4p14	UCHL1[3]	No
PARK6	AR	1p35	Serine-threonine protein kinase	Yes
PARK7	AR	1p36	DJ-1	No
PARK8	AD	12p11	Leucine rich repeat kinase 2	Yes
PARK9 (Kufor-Rakeb Syndrome)	AR	1p36	Unknown	No
PARK10	Idiopathic	1p32	Unknown	No
PARK11 (?)	AD	2q22	NR4A2	No

Source: Adapted from the Human Gene Nomenclature Database (www.gene.ucl.ac.uk/nomenclature/).
[1] DNA-based testing.
[2] SNCA, α-synuclein.
[3] UCHL1, ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase).
AD, autosomal dominant; AR, autosomal recessive.

Mutations are generally exon rearrangements leading to deletions, duplications, triplications, or point mutations. While current estimates indicate that >90% of all *PARKIN* mutations can be identified by molecular methods,[29,30] testing is presently available only on a research basis.

PARK5, a rare autosomal dominant form of PD, may be associated with mutations in the *UCHL1* gene, although to date only a single partially penetrant missense mutation in one family has been identified, raising the possibility that this sequence alteration might be a rare genetic polymorphism.[31] Ubiquitin C terminal hydrolase L1 (UCHL1) is an enzyme of the ubiquitin C terminal hydrolase family. Abundantly expressed in brain, this enzyme is directly involved in the protein ubiquitination pathway responsible for the normal clearance and recycling of proteins from neurons. The mutant form of UCHL1 has been reported to have diminished enzyme activity, resulting in impaired protein clearance through the ubiquitin-proteosome pathway.[28] PARK6 is associated with mutations in the serine-threonine protein kinase gene (PINK1).[3]

Mutations (deletion and point mutations) in the recently identified *DJ1* gene are associated with PARK7, another autosomal recessive form of PD. Although the specific function of this newly identified gene is unknown, preliminary data suggest involvement in the cellular oxidative stress response.[32] PARK8 is associated with mutations in the leucine rich repeat kinase 2 gene.[3]

The most recently characterized PD gene, *NR4A2*, is associated with yet another autosomal dominant form of the disease (PARK11). *NR4A2*, encoding a member of nuclear receptor superfamily, is involved in the differentiation of nigral dopaminergic neurons, suggesting that mutations within the gene can result in dopaminergic dysfunction. Both a deletion and a missense mutation in exon 1 of *NR4A2* have been identified in multiple symptomatic members of ten kindreds segregating with an autosomal dominant PD phenotype.[33]

Clinical Utility of Testing

Testing for PARK1, 2, 6, and 8 are available clinically. Within the context of a high degree of clinical suspicion and the documentation of a family history of PD, molecular testing has demonstrable utility. Confirmation of a pathologic mutation in a proband will establish a diagnosis and clarify the mode of inheritance such that, theoretically, both presymptomatic testing for appropriate family members and prenatal testing for at-risk pregnancies could be offered.

Available Assays

Testing for patients with suspected *SCNA* gene mutations can be performed by conventional PCR-RFLP analysis of the mutation-containing exons. Testing for *PARKIN* mutations generally necessitates the use of a semiquantitative PCR methodology to detect heterozygous exonic rearrangements (deletions, duplications, and triplications), which would not be detected by conventional nonquantitative PCR or direct sequencing.[30]

Interpretation of Results

The identification of a pathologic mutations in a symptomatic proband is diagnostic.

Laboratory Issues

No commercial in vitro diagnostic test kits nor formal proficiency testing are currently available for PARK1.

AMYOTROPHIC LATERAL SCLEROSIS

Molecular Basis of Disease

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by progressive loss of function of both upper and lower motor neurons (UMN, LMN) of the brain and spinal cord. The disease is characterized clinically by the presence of deficits in UMN and LMN function. UMN deficit signs generally include hyperreflexia, extensor plantar response, and increased muscle tone. LMN deficit signs include weakness, muscle wasting and cramping, and fasciculations. While 90% of ALS cases occur sporadically, the remaining 10% of patients have familial forms of the disease, which can be classified based on the mode of inheritance (Table 15-4).

Only ALS1 and ALS2 have been characterized at the molecular and protein levels. The copper-zinc superoxide dysmutase gene (*SOD1*) encodes superoxide dysmutase (SOD1), a metalloenzyme consisting of 153 amino acids with both copper and zinc binding sites. SOD1 catalyzes the conversion of superoxide anions to hydrogen peroxide and

Table 15-4. Molecular Classification of Amyotrophic Lateral Sclerosis (ALS)

Locus	Inheritance	Chromosome	Gene Product	Clinical Test[1]
ALS1	AD	21q22.1	SOD1[2]	Yes
ALS2	AR	2q33	Aslin	Yes
ALS3	AD	18q31	Unknown	No
ALS4	AD	9q34	Unknown	No
ALS5	AR	15q15	Unknown	No
ALS-FTD[3]	AD	9q22	Unknown	No

Source: See GeneTests (www.genetests.org).
[1] DNA-based testing.
[2] SOD1, superoxide dysmutase (Cu-Zn).
[3] ALS-FTD, ALS with frontotemporal dementia.
AD, autosomal dominant. AR, autosomal recessive.

molecular oxygen and, as such, is thought to have a protective function in the cell by preventing oxidative damage caused by the accumulation of free radicals. More than 90 mutations have been reported in the *SOD1* gene, with one, A4V, accounting for approximately 50% of all mutations found in North American families. Approximately 20% of patients with familial ALS and approximately 3% of patients with sporadic ALS have *SOD1* mutations (associated with ALS1).[34]

The gene product for ALS2, or autosomal recessive juvenile-onset ALS, recently has been identified and characterized. The protein, alsin, is predicted to encode a 184 kDa protein containing 1,657 amino acids. Analysis of protein sequence homologies suggests that alsin may belong to a family of GTPase regulator proteins. All ALS2 families studied to date carry deletion mutations that lead to frameshifts, generating premature stop codons.[35,36]

Clinical Utility of Testing

Within the context of a high degree of clinical suspicion and the documentation of a positive family history, DNA diagnostic testing for ALS1 and ALS2 has demonstrable utility. Confirmation of a pathologic mutation in a proband will establish a diagnosis and clarify the mode of inheritance such that both presymptomatic testing for appropriate family members and prenatal testing for at-risk pregnancies could be offered.

Available Assays

Due to allelic heterogeneity for both ALS1 and ALS2, clinical testing for ALS1 requires the use of direct DNA sequencing.

Interpretation of Test Results

The identification of a pathologic *SOD1* mutation in a symptomatic proband is diagnostic. However, as test sensitivity is estimated to be no greater than 50%, negative results must be interpreted with caution. While both presymptomatic as well as prenatal testing are theoretically possible in mutation-positive kindreds, the existence of incomplete or reduced penetrance makes pretest counseling difficult.

Laboratory Issues

No commercial in vitro diagnostic test kits are available for ALS. Several cell lines and at least one purified genomic DNA sample from a family with autosomal dominant ALS can be obtained from the Coriell Cell Repositories (Camden, NJ; http://ccr.coriell.org/); however, the mutation segregating in the family has not been characterized. Formal proficiency testing for ALS1 currently is not available.

DYSTONIA

Molecular Basis of Disease

Although they are not strictly considered a neurodegenerative disease, clinical and molecular descriptions of the dystonias have historically been found in texts describing neurodegenerative disorders. With the recent neuropathological descriptions associated with DYT3 (X-linked dystonia-parkinsonism, or "Lubag" form of the disease) and DYT14,[37,38] the discussion of the dystonias in this chapter becomes even more justified. The dystonias represent a clinically heterogeneous group of disorders characterized by sustained involuntary muscle contractions leading to repetitive twisting movements and abnormal postures. At least 14 forms of dystonia (Table 15-5) can be distinguished on a genetic basis,[37,39] and genes for three of the dystonias (DYT1, DYT5, and DYT11) have been identified.[37,39] DYT1, also known as early-onset primary dystonia, is inherited in an autosomal dominant manner and is usually associated with reduced penetrance. Essentially all patients with DYT1 (>99.9%) have a 3 bp deletion (GAG) in the *DYT1* gene. A second mutation (an 18 bp in-frame deletion in exon 5 of *DYT1*) has been described in one patient with early-onset dystonia and myoclonic features.[40] The *DYT1* gene localizes to chromosome 9q34 and encodes a 332 amino acid protein called torsinA. Although its function remains unknown, torsinA shares sequence homologies with the AAA super-

Table 15-5. Molecular Classification of the Dystonias

Locus	Inheritance	Chromosome	Gene Product	Clinical Test[1]
DYT1	AD	9q34	TorsinA	Yes
DYT2	AR	Unknown	Unknown	No
DYT3	X-linked	Xq13.1	Putative DYT3 protein	Yes
DYT4	AD	Unknown	Unknown	No
DYT5	AD	14q22.1	GCH1[2]	Yes
	AR	11p15	TH[3]	No
DYT6	AD	8p21	Unknown	No
DYT7	AD	18p11.3	Unknown	No
DYT8	AD	2q25	Unknown	No
DYT9	AD	1p21	Unknown	No
DYT10	AD	16p11	Unknown	No
DYT11	AD	7q21	ε-Sarcoglycan	No
	AD	11q23	Dopamine receptor D2	No
DYT12	AD	19q13	Unknown	No
DYT13	AD	1p36.13	Unknown	No
DYT14	AD	14q13	Unknown	No

Sources: Adapted from [31, 33].
[1] DNA-based testing.
[2] GCH1 (GTP cyclohydrolase I).
[3] TH (tyrosine hydroxylase).
AD, autosomal dominant; AR, autosomal recessive.

family of ATPases[37] and is thought to have a role in several cellular functions including protein folding and degradation, membrane trafficking, vesicle fusion, cytoskeletal dynamics, and physiological responses to stress.[37]

DYT5, or dopamine-responsive dystonia (DRD), is associated with mutations in two different genes. Homozygous as well as compound heterozygous mutations in the tyrosine hydroxylase (*TH*) gene at 11p15.5 are associated with the rare autosomal recessive form of DRD. The more common autosomal dominant form of DRD is associated with mutations in the GTP cyclohydrolase I (*GCH1*) gene, which maps to 14q22.1. Both enzymes are involved in the bio-synthetic pathway for the neurotransmitter dopamine. Recently a third DRD locus (DYT14) has been mapped to chromosome 14q13 but the specific gene and its protein product are not known.[37]

DYT11, or myoclonus-dystonia, is associated with loss-of-function mutations in the ε-sarcoglycan gene at 7q21 in a large number of families and represents a major locus for myoclonus-dystonia. However, the concurrent finding of a single myoclonus-dystonia family segregating a missense mutation in the D2 dopamine receptor (*DRD2*) gene that maps to 11q23, and the identification of linkage of another large myoclonus-dystonia family to a locus on chromosome 18p11, suggests some degree of genetic heterogeneity for this form of dystonia.[37]

Clinical Utility of Testing

Clinical molecular testing is presently available for only DYT1, DYT3, and the *GCH1*-linked form of DYT5.

Available Assays

Molecular testing for DYT1 is routinely performed by PCR-RFLP analysis of the GAG-containing *DYT1* exon (Figure 15-5). Determination of pathologic mutations in *GCH1* requires direct DNA sequencing of the gene.

Interpretation of Test Results

Essentially all patients with typical early-onset dystonia (DYT1) carry the GAG deletion on one allele, thus the test sensitivity approaches 100%. In contrast, the specificity of the assay is 60% to 70%, because DYT1 is inherited as an autosomal dominant trait with reduced penetrance (30% to 40%). Counseling of the parents of the proband should include an evaluation of their clinical status and consideration of molecular testing to determine parental origin of the mutation. The risk of inheriting a *DYT1* allele from a proband is 50%; however, the probability that the gene carrier will become symptomatic is estimated to be 30% to 40%. Prenatal testing is clinically available for fetuses at 50% risk of inheriting a *DYT1* allele, once the presence of the mutation has been confirmed in the family. New mutations, although rare, have been reported, but the mutation rate is unknown.

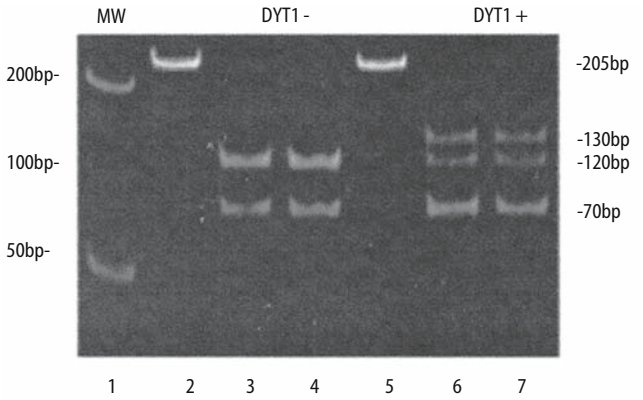

Figure 15-5. PCR-RFLP analysis of the *DYT1* GAG deletion mutation associated with autosomal dominant early-onset torsion dystonia (DYT1). DNA was amplified with the 6419/H48 primer pair[21] and the resulting 205 bp amplicon digested with *Bse* RI. The presence of the GAG deletion mutation results in the loss of a *Bse* RI site on the mutant allele and the generation of a novel 130 bp fragment in addition to the 120 bp and 70 bp fragments generated from the normal allele. Lane 1, 100 bp ladder G2101 (Promega, Madison, WI); lane 2, undigested amplicon; lanes 3 and 4, *Bse* RI digests from two *DYT1* negative patients; lane 5, undigested amplicon; lanes 6 and 7, *Bse* RI digests from two *DYT1* positive patients (note the presence of the novel 130 bp fragment).

Unlike in DYT1, more than 85 mutations have been identified in the *GCH1* form of DYT5 and, on average, approximately 30% to 40% of clinically symptomatic patients will not carry a pathologic mutation in the coding region or intron-exon boundaries of the gene. As such, clinical testing (direct sequencing) is usually limited to diagnostic testing in a proband with the knowledge that testing sensitivity may reach only 60%. If a mutation is detected, carrier testing or prenatal testing or both can be considered for family members. Counseling for the offspring and siblings of a molecularly-confirmed proband is also complicated by the influence of gender-related reduced penetrance and the existence of new mutations.[42]

Laboratory Issues

Currently no commercial in vitro diagnostic test kits are available for DYT1, DYT3, or DYT5. DYT1 DNA can be obtained from the Coriell Cell Repositories (Camden, NJ; http://ccr.coriell.org/). Currently, no formal proficiency testing is available.

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