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Diagnosis of Huntington Disease

Russell L. Margolis[1*] and Christopher A. Ross[2]

Background: **Huntington disease (HD) is a rare, progressive, and fatal autosomal dominant neurodegenerative disorder, typically of adult onset.**
Methods: **We reviewed the literature concerning the molecular diagnosis of HD.**
Results: **The discovery of the genetic etiology of HD, a trinucleotide expansion mutation on chromosome 4p, has led to the development of increasingly reliable and valid diagnostic tests that can be applied to symptomatic patients, individuals at risk for HD but currently asymptomatic, fetuses, and embryos. However, the unstable nature of the HD mutation, the lack of effective treatments for HD, the mid-adulthood age of disease onset, and the existence of disorders with the same clinical presentation but different etiology all complicates diagnostic testing.**
Conclusion: **Conscientious laboratory work, knowledgeable interpretation of genetic test results, and the availability of pre- and posttest counseling are essential components of HD diagnosis.**

Huntington disease (HD) is a rare, progressive, and fatal autosomal dominant neurodegenerative disorder, typically of adult onset, that has captured the imagination of the scientific and medical community far in excess of its direct impact on public health. The interest in the disorder began with George Huntington's classic description in 1872 *(1)*, in which he clearly described both the familial nature of the disorder and the clinical triad of movement abnormality, emotional disturbance, and cognitive impairment that still provide the basis for the clinical diagnosis of HD. In 1983, HD was the first gene mapped to a chromosomal locus by use of anonymous markers *(2)*, a process that pushed forward the field of linkage analysis and provided the molecular tools for predictive genetic testing by linkage analysis *(3)*. After a 10-year struggle, during which fundamental strategies of positional cloning were developed, the causative mutation, a CAG trinucleotide repeat expansion, was identified *(4)*. Since then, HD research and clinical practice have exerted a strong influence on the approach to phenotype-genotype relationships, the practice of genetic testing (and especially presymptomatic testing), and the understanding of the pathogenesis of neurodegeneration. This review will focus on the diagnosis of HD, with an emphasis on the interface between laboratory and clinical practice.

[1] Laboratory of Genetic Neurobiology, Division of Neurobiology, Departments of Psychiatry and Neurology, and Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287.
[2] Laboratory of Molecular Neurobiology, Division of Neurobiology, Departments of Psychiatry, Neurology, and Neuroscience, and Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287.
*Address correspondence to this author at: Meyer 2-181, 600 N. Wolfe St., Baltimore, MD 21287. Fax 410-955-8233; e-mail rmargoli@jhmi.edu.
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Clinical Presentation of HD

The prevalence rate of HD in the US and most of Europe is ~5 cases per 100 000 individuals *(5, 6)*, with small pockets of much higher prevalence. The prevalence is much lower in Japan, China, Finland, and Africa *(6)*. Age of onset varies markedly (Fig. 1), typically occurring between the ages of 35 and 50 but varying from early childhood to >80. The course is relentlessly progressive, with death usually 15–20 years after disease onset.

HD onset is defined by the beginning of motor symptoms, and most often the initial complaint that leads patients to seek medical attention is "clumsiness", "tremor", "balance trouble", or "jerkiness". The primary involuntary movement abnormality, and often the earliest symptom, is chorea or choreoathetosis, continuous and irregular writhing and jerking movements. The limbs and trunk are most prominently affected, but respiratory, laryngeal, pharyngeal, oral, and nasal musculature may also be involved. Abnormalities of voluntary movement, although usually less striking than chorea, are more associated with functional disability. Frequent findings include impaired visual tracking; slow, poorly coordinated, arrhythmic fine motor movements; dysarthria and dysphagia; rigidity; and ataxia *(6–8)*.

Cognitive abnormalities usually begin at about the same time as movement abnormalities and progress in tandem with the loss of voluntary movement capacity. As in other subcortical dementias, aphasia and agnosia are less evident than in Alzheimer disease, whereas cognitive speed and efficiency are relatively impaired *(9)*. However,

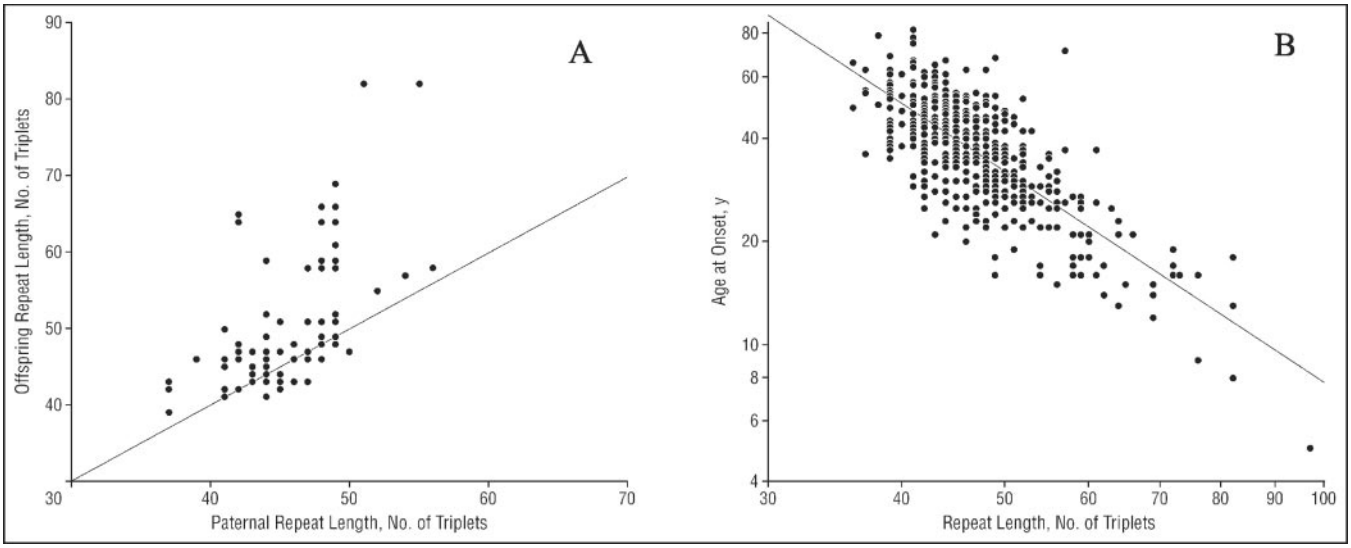

Fig. 1. Genetic basis for anticipation in HD.

Data are from the Baltimore Huntington's Disease Center. (*A*), tendency of repeat length to increase with paternal transmission of HD. Points *above* the *diagonal line* represent cases in which the repeat length increased during transmission from father to child [n = 84 pairs; mean (SD) increase of repeat length, 4.2 (0.8) triplets]. (*B*), correlation of repeat length with age at onset in HD. As repeat length increases, age at onset of disease decreases (n = 480; $r^2 = 0.57$). *y*, years. Figure reprinted from Margolis et al. *(41)* with permission of American Medical Association Press.

as the disease progresses, the dementia becomes more global. As many as 80% of HD patients develop some form of noncognitive psychiatric disorder within 10–15 years of disease onset *(8, 10)*. Mood disorders and personality changes (often manifest as irritability or apathy) are common, and the suicide rate may be as high as 5%. Fortunately, the psychiatric manifestations of HD are often responsive to treatment.

Gross pathology of HD is limited to the brain, with atrophy most prominent in the caudate, putamen, and cerebral cortex. Brain weight may be reduced by as much as 25–30% in advanced cases. The basal ganglia atrophy is readily visible by magnetic resonance imaging scan and progresses over time *(11)*. Gross cortical atrophy is also readily detectable on magnetic resonance imaging, and increasingly sophisticated volumetric analysis has demonstrated early and progressive changes in the cortex *(12)*. Microscopically, selective neuronal vulnerability is even more prominent, with loss of medium spiny neurons in the caudate and putamen *(13)* and large neurons in layers III, IV, and V of the cortex *(14)*. An especially intriguing finding is the presence of intranuclear inclusion bodies, consisting of amyloid-like fibrils that contain mutant huntingtin, ubiquitin, synuclein, and other proteins *(15, 16)*.

HD Genetics

An understanding of HD genetics is critical to interpreting the results of HD genetic tests. HD is one of nine disorders, all neurodegenerative diseases, caused by CAG repeat expansions that give rise to protein products with expanded polyglutamine tracts *(17)*. Each disease in this group is caused by an expansion in a different gene, and the genes have little in common with each other except for the presence of the CAG repeat. In the case of HD, the repeat is in exon 1 of a gene termed huntingtin (originally known as IT-15) located on chromosome 4p16.3 *(4)*. The range of repeat length in the unaffected population is 6–35 triplets. Repeats longer than 35 are considered expanded, and no individual with a repeat length <36 triplets has been convincingly diagnosed with HD. Although repeats <27 triplets in length are transmitted stably during meiosis, repeats in the range of 27–35 triplets will rarely expand to the disease range. Repeats 36–39 triplets in length are considered variably penetrant, such that the probability of developing the disease by late-life is not 100% and may be ~50% for repeats of 36 or 37 triplets *(18)* (Table 1).

Once in the disease range, repeat length is unstable during vertical transmission. The bias is toward longer repeats, at times markedly longer, in paternal transmission (Fig. 1A) *(19)*. Concomitantly, the age of disease onset is inversely correlated with repeat length (Fig. 1B) *(20, 21)*. Together, these two factors explain the phenomenon of anticipation *(22)*, in which age of onset tends to decrease in successive generations; on average, affected children will develop the disease ~8 years earlier than their affected father. The clinical manifestation of anticipation can be dramatic; at times the children of HD-gene carriers will develop the disease before their parents.

Table 1. Repeat length interpretation.

Repeat length in triplets	Interpretation
<27	Normal
27–35	May rarely expand; do not cause HD
36–39	Variable penetrance
>39	Fully penetrant

Repeat length may also decrease during vertical transmission, occasionally falling below the disease threshold.

Differential Diagnosis

The differential diagnosis of HD is important in the evaluation of clinically affected individuals who do not have the HD mutation and in redirecting attention toward HD in cases originally presenting as another disorder. Tables 2 and 3 provide a list of the more frequent hereditary and nonhereditary causes of HD-like disorders *(23)*. Specific treatment exists for several of these conditions, ranging from a change in medicines in drug-induced cases to chelation treatment in Wilson disease; therefore, a search for a definitive diagnosis is essential. Although Tables 2 and 3 emphasize chorea, the most frequent and obvious manifestation of HD, many other diseases with parkinsonian features, including Parkinson disease itself and multisystem atrophy, could be considered in the differential diagnosis of the more rigid variant of HD.

Table 3. Nonhereditary disorders that may resemble HD.[a]

Etiology	Comments
Tardive dyskinesia	Common, especially secondary to antipsychotic medicines
Sydenham chorea	Post-streptococcal; in 20% cases of rheumatic fever; children
Systemic lupus erythematosus	Chorea in 1–7%
Neurosyphilis	Usually concomitant HIV infection
Hyperthyroidism	Chorea in 2% of cases; usually young-middle age adults
Drug-induced chorea	Lithium, antiepileptic agents, stimulants, L-dopa, estrogen
Pregnancy	Rare; increased risk with history of Sydenham chorea
Polycythemia vera	Chorea in 0.5–5% of cases
Senile chorea	Late onset; may be clinically indistinguishable from late-onset HD
Basal ganglia lesions	Strokes, infections, tumors

[a] Note that genetic factors are important in many of these conditions, although none is strictly a Mendelian disorder.

basic laboratory evaluation

The discovery of the HD gene immediately led to the development of relatively simple PCR-based assays to determine the presence of the mutant allele *(24)*. Initially, the CAG repeat length was calculated from the length of a PCR product that contained both the CAG repeat and the adjacent CCG and CCT repeats, assuming that the CCG repeat contained seven triplets and the CCT repeat contained two triplets *(25)*. Subsequently, it was demonstrated that the CCG repeat varies between 7 and 12 triplets in length *(26–28)* and that the CCT repeat following the CCG repeat *(29)* can be either 2 (common) or 3 (rare) triplets in length. To prevent overestimating the length of the CAG repeat, of some importance in routine diagnostic testing and of critical importance in presymptomatic testing *(30)*, it is now recommended that laboratories use a PCR assay in which the product contains only the CAG repeat *(28, 31)* (Fig. 2).

Table 2. Hereditary disorders resembling HD.

Disease	Comments
Dominant disorders	
Denatatorubral-pallidoluysian atrophy (DRPLA)	More common in Japan than US or Europe; CAG repeat expansion in atrophin-1
Huntington disease-like 2	Clinically and pathologically indistinguishable from HD; almost exclusively African ethnicity; CTG expansion in junctophilin-3; may be relatively common in S. Africa
Benign hereditary chorea	Childhood onset; other neurologic signs may exist; occasionally progressive; linkage to chromosome 14q
Fahr disease (idiopathic basal ganglia calcification)	Basal ganglia calcification on neuroimaging; linkage to chromosome 14q
Hereditary Creutzfeldt–Jakob disease	15% of Creutzfeldt–Jakob disease cases are dominant; characteristic electroencephalographic finding and spongiform neuropathology; *PrP* gene mutation on chromosome 20p
Spinocerebellar ataxia (SCA) 17	Expansion of CAG repeat in *TBP* gene on 6q27; chorea with cerebellar ataxia
Other spinocerebellar ataxias	Primarily SCA2 (CAG expansion in *SCA2* gene) and SCA3 (Machado–Joseph disease; CAG expansion in *MJD* gene); may occur in other types
Recessive disorders	
Ceroid neuronal lipofuscinoses	Lysosomal storage disorders; mostly childhood onset; mutations in eight different genes (*CLN1–CLN8*); adult form may be dominant or recessive
Pantothenate kinase-associated neurodegeneration	Abnormal iron accumulation; "eye of the tiger" sign on magnetic resonance imaging; 50% cases from *PANK2* mutations
Wilson disease	Abnormal copper metabolism; liver toxicity; Kayser–Fleischer rings; mutations in *ATP7B*
Other hereditary disorders	
Mitochondrial disorders	Multiple types; maternal inheritance
Neuroacanthocytosis	Several conditions characterized by acanthocytes; chorea-acanthocytosis is recessive, mutation in *chorein*; McCleod syndrome is X-linked, mutation in *Xk* gene; dominant forms also exist

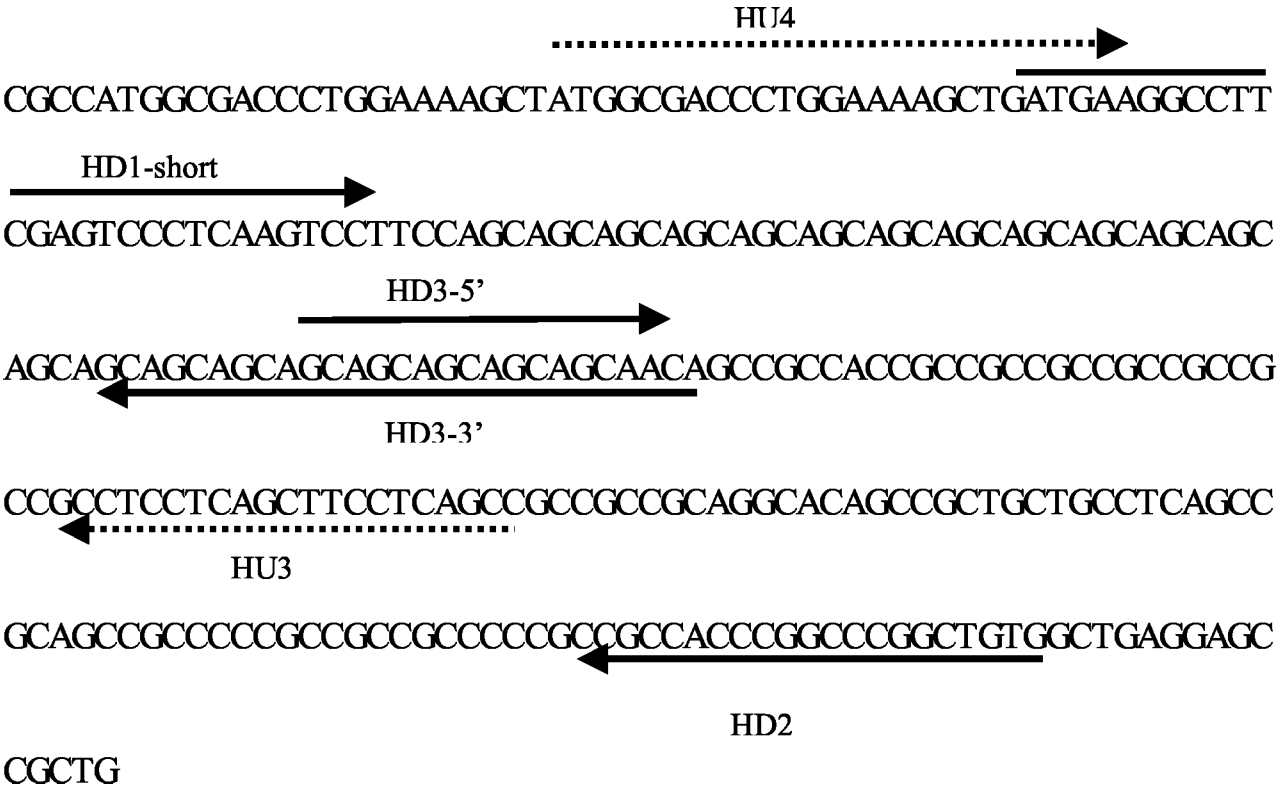

Fig. 2. HD repeat region and primer pairs.

The CAG repeat is amplified using primers HD1-short and HD3-3′. *HD1-short* is the standard PCR primer developed for this assay except that the 3′-most base was removed to avoid loss of annealing in those cases with a C-to-G polymorphism of the base immediately preceding the repeat (see Fig. 3). The CCG/CCT repeat is amplified using HD3-5′ and HD2. The entire repeat region is amplified using HD1-short and HD2 or, to maximize independence of assays, primers HU4 and HU3. Other possible primer pairs and assay modifications have been reported *(34)*.

The size of the PCR product is evaluated with polyacrylamide gel electrophoresis. The product length is detected by use of a radiolabeled primer to generate a radiolabeled product, a radiolabeled $(CAG)_n$ or $(CTG)_n$ oligonucleotide to probe a Southern blot of the gel, or a fluorescently tagged primer to generate fluorescent products detected by an automated genotyping system (Applied Biosystems, Inc). Capillary electrophoresis is now used in place of polyacrylamide gel electrophoresis with more recent ABI technology; the change from gels to capillaries necessitates recalibration of the assay because products with expanded repeats may not run at identical speeds in the two systems. Control samples of known repeat length are essential, especially in interpreting borderline expansions, because repeat expansion may slightly alter the electrophoretic mobility relative to standard size markers. Even with ideal controls, however, it is unlikely that repeat lengths can be reliably assigned without an error of a least ± 1 triplet. For longer expansions, the ability to precisely define repeat length is even less reliable, although this imprecision has little clinical consequence. Another source of variability in determining repeat length is the presence of multiple bands (on gels) or peaks (automated genotyping), instead of single bands or peaks, for expanded alleles. This represents a combination of PCR artifact and mosaicism in lymphocytes. Standard practice is to use the most prominent peak or band as the reported repeat length, but at times distinguishing among the bands/peaks is arbitrary. It is therefore often advantageous for the same laboratory to test multiple members of a family, especially in presymptomatic diagnosis, to directly compare allele lengths.

Laboratory Evaluation of Apparent Homozygosity

The detection of a single repeat is a potential source of diagnostic confusion. Usually the result accurately reflects repeat length homozygosity. By taking advantage of the original PCR assay for repeat length that includes both CAG and CCG/CCT repeats, it is possible to confirm CAG repeat length homozygosity *(31)*. With this version of the assay, two different PCR products will be discernible if the length of the CCG repeat (or, rarely, the CCT repeat) is heterozygous. If necessary, the length of the CAG repeat length can be confirmed by a second PCR that amplifies only the CCG (and CCT) repeat *(28, 32)*.

Detection of a single PCR product may also stem from failure of the PCR assay. One possibility is failure to amplify a very long expansion. This should be suspected in juvenile-onset cases. In this case, a genomic Southern analysis will reliably demonstrate the expansion, although the exact length of the expansion may be difficult to determine. The standard CAG-only assay may also fail

to amplify an allele because of a polymorphism that prevents proper primer annealing. Five such polymorphisms have been detected to date *(32–34)*, and it is possible that others exist (Fig. 3). The frequency of these polymorphisms may be as great as 1% *(34)*. On the basis of these polymorphisms, it seems reasonable to use a primer one nucleotide shorter than the standard forward CAG-only primer and to confirm apparent CAG repeat length homozygosity with an alternative set of primers. A genomic Southern analysis will again yield a definitive result.

"Sporadic" HD

Recent evidence suggests that the incidence of genetically confirmed HD in symptomatic individuals with no known family history of HD may be as high as 8% of all individuals with HD. This in part reflects rare de novo expansions from nonpenetrant but unstable repeats in the 27–35 triplet range. Other factors include anticipation (age of onset in child before parent), early death or misdiagnosis of affected parent, adoption, and false paternity. Therefore, the lack of a family history is not sufficient evidence to exclude the diagnosis of HD.

Presymptomatic Testing: Special Issues

Presymptomatic testing for HD has received extensive consideration *(35)*, and the experience and protocols developed for HD serve as prototypes for presymptomatic testing of other disorders. Detailed guidelines have been established *(36)*. Pretesting genetic counseling is imperative so that the at-risk individual can make a knowledgeable decision about the risks and benefits of completing the testing protocol. Hundreds of at-risk individuals have now completed presymptomatic testing. The key findings of this experience have been that (*a*) only a minority of individuals desire testing, and (*b*) few individuals are significantly traumatized by receiving the results.

Given the consequence of an error, many laboratories have opted to use modified testing protocols for presymptomatic testing. Some laboratories require two separate blood samples, submitted at different times. Results from the two samples are compared, minimizing the chance of a sample mix-up or technical error. Other laboratories perform PCR and Southern analyses on all samples and establish that the results from the two tests are consistent with each other before reporting the results. Our laboratory attempts to obtain HD repeat results from both parents or, at the very least, one affected relative. This confirms that the disease in affected family members is HD and, by comparing repeat length of both alleles between parents and offspring, provides a check against sample labeling errors. We perform two different PCR assays and check automated genotyping results against standard agarose gels. It is in presymptomatic testing that amplification of a single allele, as discussed above, is most problematic. In addition, given that the variability in determining repeat length in most laboratories is at least ± 1 triplet, interpreting the implications of a repeat at the margin of the disease range is often difficult.

Equally problematic is a clear negative result (two unexpanded alleles) in the absence of a genetically confirmed family history of HD. In such cases, especially when details of the family history are not available, it is possible that the disease in the family of the at-risk individual is not HD. The recent finding that Huntington disease-like 2 is both clinically and pathologically indistinguishable from HD but is caused by a different mutation *(2, 37)* demonstrates that even pathologically diagnosed HD does not prove that the familial disease is caused by the HD mutation. Genetic counselors and other clinicians working with at-risk individuals need to recognize the limitations of a negative HD presymptomatic test in cases where the HD mutation has not been demonstrated in a family member.

Another complication in presymptomatic testing arises when the adult grandchild of an individual with HD desires presymptomatic testing whereas the middle-aged child of the affected individual does not want to know his or her gene status. One option is termed exclusion testing,

Normal:	TTC (CAG)n CAG CAG CAA CAG CCG CCA (CCG)n
1. Gellera et al. *(32)*	TTC (CAG)n CAG CAG CA**G** CAG CCG CCA (CCG)n
2. Margolis et al. *(33)*	TT**G** (CAG)n CAG CAG CAA CAG CCG CCA (CCG)n
3. Margolis et al. *(33)*	TTC (CAG)n CAG CAG CAA CAG CCG CC**G** (CCG)n
4. Yu et al. *(34)*	TTC (CAG)n CA**A** CAG CAA CAG CCG CCA (CCG)n
5. Yu et al. *(34)*	TTC (CAG)n CAG CA**A** CAA CAG CCG CCA (CCG)n

Fig. 3. Polymorphisms in the huntingtin repeat region.

The five depicted polymorphisms (*bold, underlined bases*) potentially alter annealing of one of the standard primers used for CAG repeat amplification.

in which it is determined, by genotyping markers linked to the HD gene, whether the grandchild received an allele from the affected grandparent or the unaffected spouse of that grandparent. If an allele is inherited from the unaffected grandparent, the risk of HD is low (although, because of rare meiotic crossovers between the marker and the HD gene, not 0%). If an allele is inherited from the affected grandparent, the risk of HD is 50% because it is not known whether the allele inherited from the affected grandparent is the allele with or without the mutation. In the process, no information is obtained that would change the risk of the child of the affected individual.

Direct tests of the length of the HD mutation and exclusion testing have been successfully applied in utero and before implantation of embryos generated by in vitro fertilization [preimplantation genetic diagnosis (PGD)]. Test interpretation is much the same as in other forms of presymptomatic testing, with the exception that PGD requires preparation of DNA from a single cell. If the at-risk prospective parent does not want to know his or her gene status, PGD can be performed without disclosing to the at-risk parent the result of the HD test *(38, 39)*. This approach places great burdens of confidentiality on the genetic testing team, requires unnecessary cycles of in vitro fertilization and preimplantation testing for noncarriers, and, if all embryos carry the HD mutation or no embryos are suitable for implantation, may require a sham implantation procedure to preserve the blindness of the parent. On the other hand, parents should probably be advised not to consider in utero exclusion testing unless they are prepared to terminate the pregnancy for a positive test; otherwise, the risk that the child will develop HD, initially 50%, will increase to nearly 100% if the at-risk parent subsequently develops HD. As an alternative, exclusion testing has now been developed for PGD that involves use of two markers in a duplex PCR protocol *(40)*.

Conclusion

With the discovery of the mutation that causes HD, definitive molecular diagnosis of HD is now possible. Laboratories performing HD testing, and clinicians interpreting the results of testing, must be aware of potential pitfalls. The most prominent concerns include failure or inaccuracy of the standard testing protocol, ambiguous clinical implications of repeat expansions in the nonpenetrant range, and the ambiguity of negative presymptomatic testing results in the absence of information about the molecular diagnosis of affected family members. Fortunately, methods have already been developed to overcome most of the technical difficulties encountered in the assay, and substantial data now exist to serve as a guide in interpreting tests results.

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