

HUNTINGTON DISEASE

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Huntington disease (HD) is a progressive neurodegenerative disorder with an established genetic origin and symptoms that are referable to specific regions of brain disease. Cellular and molecular techniques are rapidly elucidating the pathogenesis of the disorder and are leading to approaches designed to develop rational treatments. Thus, HD serves as a model for the future study of those psychiatric disorders in which abnormal brain function is thought to arise from predominantly genetic factors.

CLINICAL FEATURES

HD can be described as a triad of motor, cognitive, and emotional disturbances (1,2). Symptoms usually begin between the ages of 35 and 50 years, although the onset may occur at any time from childhood to old age. Death occurs an average of 15 to 20 years after symptoms first appear, with some patients dying earlier from falls or suicide and others surviving for 30 to 40 years (Fig. 125.1).

Movement Disorders

The *movement disorder* of HD consists of two components: *involuntary* movements and abnormal *voluntary* movements. *Chorea*, or choreoathetosis, is the movement abnormality most frequently associated with HD. It consists of continuous and irregular jerky or writhing motions. Disturbances of voluntary movement, however, are more highly correlated with functional disability and disease severity, as measured by the degree of brain disease. The disordered voluntary movements observed in HD include the following: abnormal eye movements, such as slow, hypometric saccades and catchy pursuit; uncoordinated, arrhythmic, and slow fine motor movements; dysphagia and dysarthria; dysdiadochokinesis; rigidity; and gait disturbances.

The nature of the motor symptoms changes over time. The onset is usually insidious. Early complaints include clumsiness, difficulty with balance, and jerky movements or tremor. In addition to limb and truncal movements, patients may have motor tics or chorea involving respiratory, laryngeal, pharyngeal, oral, or nasal musculature. Chorea often plateaus and even wanes in the later stages of the disease, but disturbances in voluntary movement continue to progress (3). In late-stage HD, patients typically become akinetic and largely nonverbal, with severe rigidity and joint contractures. At this point, they may have few involuntary movements except for occasional movements of the entire body, resembling myoclonic jerks, when disturbed. Difficulties with swallowing commonly lead to death in HD, either directly from suffocation or aspiration or indirectly from starvation.

When HD begins in childhood or adolescence (juvenile-onset HD), the presentation is often somewhat different, with prominent bradykinesia, rigidity and dystonia, and minimal chorea. Involuntary movements may take the form of tremors, and patients may develop seizures and myoclonus.

Cognitive Disorders

Cognitive difficulties usually begin about the same time and proceed at the same rate as the abnormal movements (4), although some patients may have considerable motor impairment with very little dementia, or the reverse. Early in the course of HD, aphasia and agnosia are usually much less obvious than in the cortical dementias such as Alzheimer disease, whereas deficits in cognitive speed and flexibility are more common. In contrast to Alzheimer disease, patients with HD seem to have trouble with retrieval rather than storage of memories. They are more apt than patients with Alzheimer disease to recognize words from a previously memorized list or to respond to other cues to help them recall information. This distinction has led to the classification of HD as a *subcortical dementia* (5). Cognitive losses accumulate progressively. Deficits in memory, visuospatial abilities, and judgment develop, and patients with late-stage

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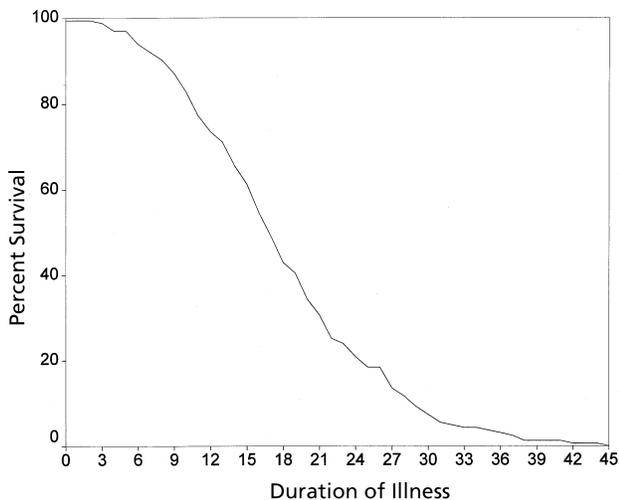


FIGURE 125.1. HD survival curve. The percentage of patients surviving as a function of years since disease onset. The curve is derived from 163 patients enrolled in the Baltimore Huntington's Disease Center with a record of both age at disease onset and age at death. (From Ross CA, Margolis RL, Rosenblatt A, et al. Reviews in molecular medicine: Huntington disease and the related disorder, dentatorubral-pallidoluysian atrophy (DRPLA). *Medicine (Baltimore)* 1997;76:305–338, with permission.)

HD demonstrate profound global impairment similar to patients with late-stage Alzheimer disease, although their paucity of speech makes assessment difficult.

Psychiatric Disorders

Patients with HD frequently develop *psychiatric symptoms*, most commonly depression, irritability, and apathy (3). The behavioral expression of these symptoms varies considerably, and it may include aggressive outbursts, impulsiveness, social withdrawal, and suicide. This aspect of HD can be devastating to both the patient and his or her family. The suicide rate alone, estimated at up to 12.7%, indicates the magnitude of the problem. Yet of all the complications of HD, the psychiatric manifestations are the most amenable to treatment.

Affective (mood) disorder is extremely common. Epidemiologic and phenomenologic evidence indicates that affective disorder in HD is a function of the brain disease itself, rather than a reaction to changes in life circumstance (2). HD-related major depression resembles the idiopathic form of major depression. Prominent symptoms include feelings of worthlessness or guilt, self-blame, changes in sleep and appetite, anxiety, anhedonia, loss of energy, hopelessness, and diurnal variation of mood with more severe symptoms in the morning. Delusions and hallucinations, when present, tend to be mood congruent: delusions of poverty, illness, or guilt; auditory hallucinations of derogatory or threatening voices. The diagnosis of major depression may

be more difficult in patients with advanced disease, but the condition is often signaled by a departure from baseline levels of activity or functional capacity.

Severe irritability is another common symptom, present in one-third of patients in the Maryland HD survey (2). Irritability and aggression may occur in patients without a prior history of a short temper, but these symptoms are more common in patients who have had these traits all their lives. Apathy may become evident at any time in the course of the disease. Once present, it tends to persist or worsen. Irritability can coexist with apathy. Either apathy or irritability may exist independently or as part of an affective syndrome.

Patients with HD occasionally develop classic obsessive-compulsive disorder, with typical symptoms such as fear of contamination or excessive hand washing. More commonly, however, patients may display an obsessive preoccupation with particular ideas or plans (e.g., obtaining cigarettes, getting a refill of coffee) and may become irritable when these requests are not honored. Rarely, patients develop a schizophrenia-like syndrome, with prominent delusions, hallucinations, or thought disorder in the absence of an abnormal mood.

Clinical Course

In summary, adult-onset HD falls roughly into three stages. Early in the disease, manifestations include subtle changes in coordination, perhaps some minor involuntary movements, difficulty thinking through problems, and, often, a depressed or irritable mood. In the middle stage, chorea usually becomes prominent, and difficulty with voluntary motor activities becomes more evident with worsening dysarthria and dysphagia. As cognitive deficits increase, the patient becomes unable to hold a job or carry out most household responsibilities. Patients with late-stage disease may have severe chorea, but they are more often rigid and bradykinetic. They are largely nonverbal and bedridden, with a more global dementia, although they retain a significant degree of comprehension.

SYMPTOMATIC TREATMENT

There are no currently accepted specific treatments to slow the rate of clinical progression of HD (6). However, symptomatic management of both movement and emotional disturbances is possible (2). A detailed handbook of HD treatment options was prepared by Rosenblatt and colleagues (6).

Treatment of Movement Abnormalities

Chorea may be a disabling symptom, leading to bruises, fractures, or falls and impairing the ability of patients to

feed themselves. Other patients find the chorea of major cosmetic concern. Treatment with high-potency neuroleptics, such as haloperidol and fluphenazine, may be indicated in such cases, but with important caveats. These medicines may exacerbate the disturbance of voluntary movement, which, as noted earlier, correlates best with functional disability. Furthermore, neuroleptics increase morbidity by making patients more rigid, sedated, and apathetic.

If pharmacologic treatment for chorea is initiated, starting doses of neuroleptics should be low, for example, 0.5 to 1 mg of haloperidol or fluphenazine per day. Doses higher than 10 mg per day of haloperidol yield little or no benefit over lower doses. If patients experience unacceptable rigidity, akathisia or dystonic reactions to high-potency neuroleptics, lower-potency agents such as thioridazine may be better tolerated. However, use of lower-potency neuroleptics increases the risk of sedation, anticholinergic side effects, and postural hypotension.

Dopamine-depleting agents such as reserpine and tetrabenazine represent another option in the treatment of chorea. Reserpine is a known cause of drug-induced depression, and the affective state of patients receiving this agent should be monitored carefully. The benzodiazepine clonazepam may also be useful in the treatment of chorea, and it may be of benefit in the later stages of the disease, when neuroleptic medication often has little effect.

Treatment of Cognitive Abnormalities

There is no known effective pharmacologic treatment for the dementia of HD. Cholinergic agents have not been systematically assessed in HD, but the rationale for using these medications is less compelling than in Alzheimer disease, because cholinergic neurons are relatively spared in HD. Patients can be instructed to jot down notes and reminders and to sequence tasks so they can concentrate on one at a time. Complex cognitive tasks should be minimized, and, as the disease progresses, questions should be framed in a choice format with the provision of frequent cues to assist recall.

Treatment of Psychiatric Disorders

Major depression in HD responds to the same treatments used in idiopathic depression. In general, depression in HD is underdiagnosed and undertreated, perhaps because of the propensity of clinicians to see it as an understandable reaction to having the disease. Although no controlled studies exist, our experience is that both tricyclic antidepressants and selective serotonin reuptake inhibitors are effective. As with any neuropsychiatric disorder, patients should be started on low doses that are slowly increased while the patient is closely monitored for adverse effects, particularly delirium. It is important to remain with a medication for a full therapeutic trial at adequate doses and blood levels.

Depressed patients should always be questioned about thoughts of suicide. When suicide is a concern, the patient should receive as few pills as possible, especially if they are to be kept in the patient's care.

The addition of antipsychotic (neuroleptic) medication is indicated as an *adjunct* to antidepressant treatment in depressed patients with hallucinations or delusions. Clozapine and other atypical neuroleptics may have the advantage over traditional neuroleptic medications, such as haloperidol, of causing fewer extrapyramidal side effects and therefore not worsening aspects of the voluntary movement disturbance. Electroconvulsive therapy is indicated for depressed patients who are refractory to treatment with medication, for patients with delusions, for those who are not eating or drinking because of their depression, or for those who are at high risk of suicide. For patients with bipolar disorder, carbamazepine, divalproex sodium, or lithium may be the initial treatment of choice; again, it is prudent to start with low doses that are gradually increased until symptoms respond, side effects make further dose increases counterproductive, or therapeutic blood levels have been reached.

In treating irritability, it is important to attempt to identify and to minimize precipitants such as hunger, pain, inability to communicate, frustration with failing capabilities, boredom, difficult interpersonal relationships, and minor unexpected changes in routine. Pharmacologic treatment can be very effective. We have had success using selective serotonin reuptake inhibitors (7) and divalproex. Sexual disorders in HD, particularly aggressive hypersexuality, can be treated with antiandrogenic medications. Obsessive-compulsive disorder in HD can be treated with standard antiobsessional agents, such as selective serotonin reuptake inhibitors and clomipramine.

DIFFERENTIAL DIAGNOSIS

The clinical features of HD are often characteristic, and the diagnosis is not difficult in a patient with a known family history, typical choreiform movements, and cognitive dysfunction. The diagnosis is less clear in patients with uncharacteristic presentations or a lack of family history (1,8). For instance, patients may present with very little chorea or with movements that are predominantly athetoid, dystonic, or even ticlike. All the affected members of a pedigree may manifest atypical features of the disorder, such as prominent brainstem involvement, a finding contributing to diagnostic confusion. Occasional patients (particularly with late onset) may have only subtle movement abnormality and relatively little cognitive disorder (1,8). Fortunately, with the availability of the HD gene test, it is now possible to establish the diagnosis of HD definitively even in patients with no family history or an atypical presentation. Most patients thought to have HD on clinical examination but who do

not have the triplet repeat expansion appear to have atypical features, more characteristic of spinocerebellar ataxias or other multisystem atrophies.

HD is now recognized as part of a family of related neurodegenerative disorders, all caused by expansions of CAG repeats encoding glutamine (9). The diseases share certain clinical features, especially ataxia and dementia, and can be confused with each other. Among these diseases, Machado–Joseph disease (MIM no. 109150) and dentatorubral pallidolusian atrophy (MIM no. 125370) are most like HD. Various other diseases may also present with HD-like symptoms, including Wilson disease, Creutzfeldt–Jakob disease, forms of ceroid neuronal lipofuscinoses, chorea with red blood cell acanthocytosis, hereditary nonprogressive chorea, paroxysmal choreoathetosis, mitochondrial disorders, corticobasal degeneration, basal ganglia calcification, forms of hereditary dystonia, Sydenham chorea, vitamin E deficiency, and cerebral vascular disease (10).

GENETIC ETIOLOGY

Discovery of the HD Mutation

HD was the first disease mapped (to chromosome 4p) using the techniques of linkage analysis with anonymous DNA probes (11). The techniques of linkage analysis were used to demonstrate that HD exhibited almost complete genetic dominance (13) and locus homogeneity (14). Many of the techniques of positional cloning, including marker development, recombination and haplotype analysis, linkage disequilibrium, physical mapping, and exon amplification, were first developed or tested during the search for the HD gene (12).

Using exon amplification and cDNA cloning, the actual gene (IT15 or huntingtin) was identified in 1993 (15). The mutation proved to be an expansion of a CAG repeat, making HD a member of a group of similar triplet repeat disorders (9, 16–18). The IT15 gene is composed of 67 exons in both mice and humans (19), and in human it is located between markers D4S127 and D4S180 on chromosome 4p16.3. It spans a genomic region of more than 200 kb and is transcribed into two versions of mRNA, varying only in the length of their 3' untranslated region. The open reading frame encodes a protein of about 350 kd with no significant homology to known proteins (15,19).

Genetic Diagnosis

Diagnosis of HD has been greatly simplified by the direct triplet repeat gene test. Previously, genetic diagnosis required cumbersome linkage analysis, impossible if family members were not available or were not heterozygous for the linkage markers. By contrast, the direct gene test, typically a single polymerase chain reaction, enables the length of the repeat in each allele to be measured. The test is highly sensi-

tive and specific, although several variations in the sequence surrounding the repeat can lead to erroneous results (20).

On a population basis, there is a clear distinction between expanded and normal length repeats in huntingtin. Repeats with fewer than 29 triplets are within the normal range. The rare repeats with 29 to 35 triplets are considered of intermediate length, prone to expansion but not in themselves of sufficient length to produce a phenotype. Repeats with 36 or more triplets are considered expansions (21).

Before the discovery of the causative expansion mutation, HD was considered 100% penetrant. However, it is now clear that *penetrance* (currently defined as the presence of signs or symptoms of HD by the age of 65 years) is less than 100% in persons carrying an allele with 36 to 40 triplets. For instance, four of seven persons who were more than 70 years old and who had a 36 triplet allele had no signs or symptoms of HD. One person with a 39 triplet allele died at the age of 95 years with no definite clinical or pathologic evidence of HD (21). Because alleles with 36 to 40 triplets are quite uncommon, the frequency of nonpenetrance is difficult to estimate reliably. However, for repeats of 36 or 37 triplets, it may be on the order of 50%.

The discovery of the HD mutation revolutionized genetic counseling of presymptomatic persons at risk of HD. Between 1983 and 1993, genetic testing was based on linkage analysis, a complex procedure requiring the cooperation of several family members. HD emerged as a model for presymptomatic genetic testing, and most testing has been carried out under careful protocols involving extensive counseling and patient education (22). Most persons, including those testing positive, state that they are relieved to know the results, and this knowledge enables them to face the future with less uncertainty. Not all patients, however, have had unequivocally positive experiences with testing (23). The central difficulty stems from the availability of a presymptomatic test for a disorder with limited therapeutic interventions (at present), a dilemma termed the *Tiresias complex* from Tiresias' words to Oedipus: "It is but sorrow to be wise when wisdom profits not" (23).

Repeat Length Instability and HD Clinical Genetics

Analysis of the triplet repeat has clarified the issue of new mutations in HD. The previous belief, that new HD mutations do not occur, was disproved with the discovery of the HD repeat expansion. The lengths of normal repeats change in fewer than 1% of intergenerational transmissions; when the length does change, it is typically by only one triplet. The frequency of changes in length increases with transmission of longer repeats and becomes appreciable for alleles with repeats of intermediate length (29 to 35 triplets). During paternal transmission, these alleles are more likely to expand than to contract, and the change, unlike in normal repeats, is often of more than one or two triplets. On occa-

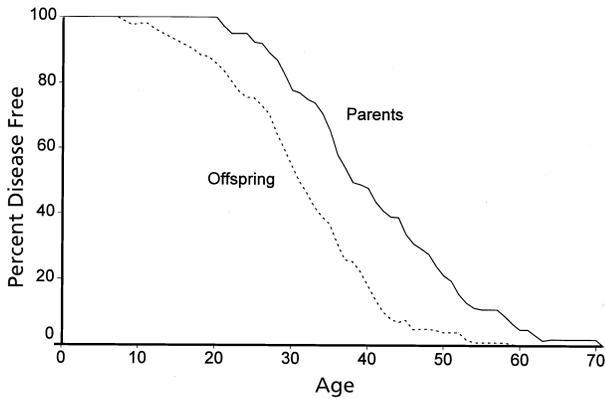


FIGURE 125.2. Anticipation in Huntington disease. The age at which affected parents and their affected children first manifest disease symptoms is depicted as a survival curve. The younger generation is affected at a substantially earlier age. Data from 61 parents and 82 children. (From Margolis RL, McInnis MG, Rosenblatt A, et al. Trinucleotide repeat expansion and neuropsychiatric disease. *Arch Gen Psychiatry* 1999;56:1019–1031, with permission.)

sion, the repeat can expand to a length sufficient to cause clinical HD. Therefore, new HD mutations do occur, arising from alleles in the intermediate range (24,25). Factors increasing instability include change of a CAA triplet adjacent to the CAG repeat into a CAG and advanced paternal age.

The nature of the HD mutation now provides a molecular basis for understanding *anticipation*, the phenomenon of increasing disease severity or decreasing age of onset in successive generations (26). Anticipation in HD has long been recognized (Fig. 125.2). In our clinical sample of affected parent-child pairs, there was no significant change in the age of onset in maternal transmission, but a mean advance of 8 years in paternal transmission. In addition, it is now clear that most patients with juvenile-onset HD arise from paternal transmission (19,27,28). Two features of the molecular genetics of HD explain the phenomenon of anticipation (29,30). First, the age of HD onset is inversely correlated with repeat length, a quite striking phenomenon (Fig. 125.3). Second, the length of the expanded triplet repeat is unstable in vertical transmission (Fig. 125.4). Paternal alleles more frequently expand than contract during transmission, whereas maternal alleles have an equal probability of expanding and contracting. Instability increases as repeat length increases (27,31). The net result, driven by paternal transmissions, is a skew toward earlier ages of onset in successive generations of a family.

The increasing length of the repeat in paternal transmission appears to arise during spermatogenesis (32). In particular, persons with longer expanded repeats (as assayed from DNA obtained from leukocytes) have dramatically variable repeat lengths in individual sperm. The variability in oocytes

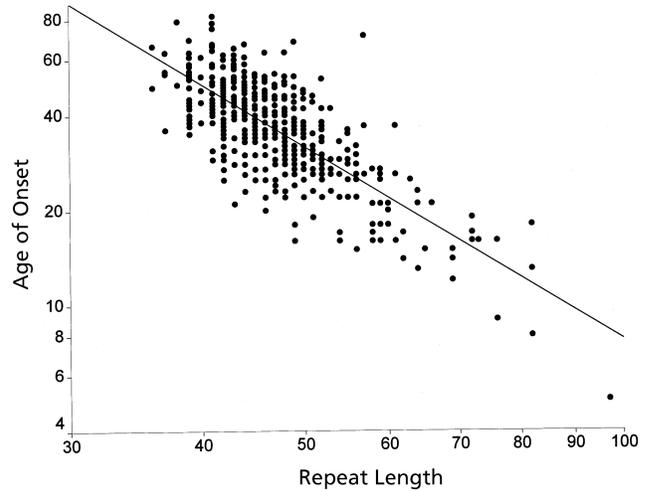


FIGURE 125.3. Correlation of repeat length with age at onset of HD. As repeat length increases, age at onset of disease decreases ($n = 480$; $r^2 = 0.57$). (From Margolis RL, McInnis MG, Rosenblatt A, et al. Trinucleotide repeat expansion and neuropsychiatric disease. *Arch Gen Psychiatry* 1999;56:1019–1031, with permission.)

appears to be less, perhaps because mature spermatocytes have undergone, on average, more cell divisions than mature oocytes. The increased likelihood of transmission of an expanded repeat as paternal age increases is consistent with this observation.

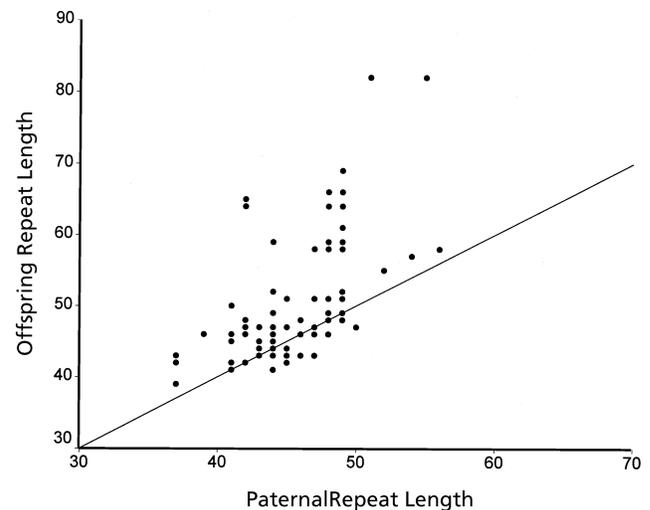


FIGURE 125.4. Increase in repeat length with paternal transmission of the HD disease allele. Points above the diagonal line represent cases in which the repeat length increased during transmission from father to child ($n = 84$ pairs; mean increase of repeat length \pm SD = 4.2 ± 0.8 triplets). (From Margolis RL, McInnis MG, Rosenblatt A, et al. Trinucleotide repeat expansion and neuropsychiatric disease. *Arch Gen Psychiatry* 1999;56:1019–1031, with permission.)

The relationship between the length of the repeat and the *rate* of clinical progression has not been resolved, although this relationship is of considerable importance in determining methods for slowing the course of HD. The rate of disease progression may be more rapid in cases with longer repeats (33), but this is not a universal finding (34). On postmortem examination, pathologic changes in cases with longer triplet repeats were more advanced than in cases with the same duration of illness but shorter repeats, a finding providing support for a correlation between repeat length and rate of progression (35). The related issue whether earlier age of onset correlates with more rapid disease progression has also not been resolved. There is no apparent correlation between repeat length and the presence of psychiatric symptoms.

PATHOLOGY

The only known pathologic changes of HD are specific to the brain, and they are characterized by striking regional selectivity of atrophy and neuronal loss (18). The most prominent atrophy is found in the caudate nucleus and putamen, which together comprise the corpus striatum within the basal ganglia. Striatal atrophy leads to hydrocephalus *ex vacuo* and marked dilatation of the lateral ventricles. In addition, there is overall atrophy of the brain. Total brain weight is reduced by 25% to 30% in advanced cases, a finding reflecting atrophy of the cerebral cortex and underlying white matter in addition to the basal ganglia.

Within the striatum, there is selective neuronal vulnerability both in the anatomic pattern of regions affected and in the particular neurons lost. Loss of neurons in the caudate and putamen shows a gradient, with early and most severe loss in the dorsal and medial regions and progressive loss of neurons in ventral and lateral regions as the disease progresses (36). There is severe loss of medium spiny projection neurons, especially those synthesizing enkephalin and γ -aminobutyric acid, but relative preservation of large and medium spiny interneurons (37,38). Neuronal loss is accompanied by reactive astrocytosis (gliosis). Other areas of the basal ganglia, especially the globus pallidus and subthalamic nucleus, also become atrophic, although less than the striatum. A 0 to 4 rating scale of gross and microscopic neurodegeneration, based primarily on changes in the caudate and putamen, has been used semiquantitatively to grade the severity of HD (39). Patients with more severe neurodegeneration have greater clinical impairment before they die, and, as more recently observed, they tend to have longer expanded repeats (35,40).

Quantitative studies of cell number and morphology have demonstrated shrinkage and loss of neurons in the cerebral cortex (41), consistent with previous evidence from gross pathology and neuroimaging studies. Large cortical neurons appear to be most severely affected, and there is

laminar specificity, with greatest loss in layer VI and significant loss in layers III and V. The neurons lost in the greatest numbers appear to project to the thalamus, whereas most neurons that project to the caudate and putamen lie in more superficial regions of layer V. In addition, the extent of cortical degeneration does not closely correlate to the severity of striatal degeneration. This set of observations indicates that the loss of neurons in the cortex does not arise simply from retrograde changes beginning in the striatum.

Whereas the atrophy and neuronal cell loss of HD have been extensively studied, less attention has focused on the morphology of the surviving neurons. Contrary to expectations, application of the Golgi metal impregnation method to study neuronal morphology in the caudate and cortex from HD cases revealed evidence, in the surviving neurons, of "regenerative" or "plastic" changes (42,43). Relative to neurons in these regions from normal brains, surviving neurons in HD cases had more dendrites, more long recurved dendrites, greater density and larger size of dendritic spines, and greater somatic area. A complete understanding of the pathogenesis of HD will need to encompass an explanation of these regenerative changes as well as neuronal death and brain atrophy.

Many of the clinical symptoms of HD correlate to pathologic features in the basal ganglia and cerebral cortex. The motor changes are believed to stem from interruption of a set of neuronal circuits interconnecting the cerebral cortex, the basal ganglia, and the thalamus. The involuntary and voluntary movement abnormalities in HD may arise from early degeneration of specific populations of medium spiny neurons. Changes in the basal ganglia also likely underlie the early and relatively mild "subcortical" cognitive changes seen in HD. The more severe global dementia seen late in the illness may relate to the widespread loss of neurons in the cerebral cortex, which does not appear to occur until relatively late in the disease.

Although it is relatively straightforward to correlate clinical symptoms with atrophy or neuronal cell loss, it is more difficult to determine the role of functional changes in neurons in the production of symptoms. In most cases, symptomatic HD is reflected in neuronal loss at autopsy. However, occasional cases arise of patients with clinically diagnosed HD but no discernible cell loss. These have been termed "grade zero" in the Vonsattel severity scale (39,44): there is no appreciable gliosis or neuronal loss. Therefore, it is possible that symptoms in these patients arise more from functional changes than from actual neuronal loss.

Degeneration is also observed, although less consistently and less prominently, in other regions of the brain, including the brainstem, the cerebellum, the lateral tuberal nucleus of the hypothalamus, the amygdala, and portions of the thalamus. The relationship between these changes and clinical features is not clear.

Studies of human postmortem HD brain tissue using antibodies directed at the N-terminus of huntingtin have

revealed small intranuclear inclusion bodies present in neurons but not in glia (45,46). Although they are present in other brain regions, inclusions are most abundant in the cortex and the caudate, and in those cell types (medium spiny neurons of the striatum) and cell layers (III, V, and VI of the cortex) most severely affected in HD. The density of inclusions is significantly correlated with the length of the CAG repeat in IT15 (47). Inclusions were not present in the brain of one presymptomatic person with the HD mutations.

The inclusions cannot be detected by antibodies directed at internal epitopes of huntingtin, a finding suggesting that huntingtin within the inclusions is abnormal, most likely truncated but possibly misfolded such that internal epitopes are sequestered. The inclusions can, however, be detected with antibodies to ubiquitin, a tag for proteins undergoing proteolytic degradation. This could mean that huntingtin within inclusions has been targeted for degradation but cannot be removed by proteolysis. Ultrastructural analysis of the inclusions indicates that they are composed of a mixture of granules, straight and tortuous filaments, and masses of parallel and randomly oriented fibrils, not enclosed by an intracellular membrane. Similar inclusions have been detected in transgenic mouse models of HD (48).

In addition, huntingtin has been found in aggregates in dystrophic neurites in HD-affected brains (45). These were present predominantly in cortical layers V and VI and appeared to be contained within neurofilament labeled axonal processes. Such dystrophic neurites may reflect dysfunction of retrograde axonal transport.

PATHOGENESIS

Certain lines of evidence indicate that the major pathogenic mechanisms of HD involve a toxic gain of function by the mutant protein; in other words, the abnormal length of the polyglutamine repeat gives huntingtin a toxic property not found in the wild-type protein. First, HD, like all the other polyglutamine repeat disorders, has a dominant mode of inheritance, which is typically the result of gain-of-function mutations. Second, the age of onset for homozygotes for the HD mutation generally is not markedly less than the age of onset for cases with only one copy of comparable repeat length (13), although this is not necessarily the case in the other glutamine repeat diseases. Third, no cases of HD or related polyglutamine disorders have been identified with deletions or point mutations in any of the causative genes. In contrast, the fragile X phenotype can be caused by a triplet repeat expansion leading to impaired transcription, a deletion, or a point mutation; all three types of mutations result in loss of normal protein function (49). Finally, mice with targeted deletions of the HD gene resulting in expression that is a small fraction of normal demonstrate

developmental abnormalities rather than a progressive neurologic disorder (50).

Neurotoxicity Models

Before the discovery of the genetic etiology of HD, animal models of HD had been generated using neurotoxins. Injections of *N*-methyl-D-aspartate–receptor agonists, such as quinolinic acid, into the striatum induce HD-like disease, with loss of medium spiny projection neurons and sparing of cholinergic and reduced nicotinamide-adenine dinucleotide phosphate diaphorase neurons (51). Peripheral injections into rodents or primates of several mitochondrial toxins, including 3-nitropropionic acid, also reproduce aspects of striatal disease found in HD (52). Other metabolic poisons cause preferential toxicity in different regions of the brain, often those regions affected in other glutamine repeat diseases.

These neurotoxin experiments suggest several pathways that could be involved in HD cell death (53). For instance, both excitotoxicity and metabolic poisoning may be mediated, in part, by damage from free radicals (54). In addition, neurotoxic stimuli can give rise to *apoptosis*, a controlled form of cell death under control of cellular machinery that plays an essential role in normal development. The process is triggered by a group of aspartate proteases termed caspases, and glyceraldehyde phosphate dehydrogenase (GAPDH) and other metabolic enzymes may also serve as initiating factors (55,56).

Studies of subjects with HD have yielded results consistent with some of these neurotoxicologic mechanisms. For instance, nuclear magnetic resonance spectroscopy suggests the presence of metabolic compromise within neurons (57). Marked biochemical defects of mitochondrial complex II and complex III activity, and moderate defects of complex IV activity, have been detected in mitochondria isolated from the brain tissue of individuals with HD. Evidence of free radical activation is also present in HD postmortem tissue (58,59).

Huntingtin Biochemistry

The *huntingtin* protein is widely expressed in both the brain and peripheral tissues (60,61). The CAG repeat, even when expanded, is translated into polyglutamine (62). Immunocytochemical and subcellular fractionation studies indicate that huntingtin is present in neuronal perikarya, dendrites, and terminals, with a generally cytoplasmic localization. Huntingtin has not been consistently detected in the nucleus. The protein appears to associate with cytoskeletal elements and intracellular vesicles, with enrichment in endosomal compartments and Golgi complex membranes (63), and it is detected at all stages of embryonic and postnatal brain development. Within the striatum, huntingtin may be enriched in the medium spiny neurons, the neuronal

population most severely affected in HD; expression level may therefore contribute to the selective vulnerability of the medium spiny neurons.

To provide clues about the normal function of huntingtin and about HD pathogenesis, an intensive effort has been devoted to finding proteins that interact with huntingtin. Interactors of particular interest include HIP1, HAP1, GAPDH, and SH3GL3 (64). Some of these proteins are directly or indirectly associated with microtubule motor proteins and intracellular vesicles, findings suggesting a role for huntingtin in cytoskeletal function or vesicular transport. The interaction of huntingtin and other proteins containing glutamine repeats with the metabolic enzyme GAPDH is of potential significance, given the possible role of GAPDH in apoptosis (65). Huntingtin also interacts with the nuclear co-repressor protein, and the strength of the interaction correlates to the length of the huntingtin glutamine repeat (66). This interaction suggests that huntingtin may have some role in transcriptional regulation, although relatively little huntingtin is detected in the nucleus of normal cells under most conditions.

Polyglutamine Biochemistry

Many proteins contain stretches of polyglutamine, and such tracts are more common than repeats of other amino acids (67). However, the normal function of glutamine repeats remains unknown. Proteins containing glutamine repeats often appear to have a role in the regulation of development and neurogenesis, and certain proteins with glutamine repeats are transcription factors (68). Glutamine-rich regions may function as factor interaction domains in transcription factors, but it is unclear whether glutamine repeats serve this or more specialized functions. The lengths of glutamine repeats tend to vary considerably in homologous genes from different species; mouse huntingtin has only seven consecutive glutamines, and the puffer fish homologue has only four.

One hypothesis for the role of glutamine repeats in human disease is based on the “polar zipper” model proposed by Perutz (69). He suggested that two antiparallel β strands of polyglutamine can be linked together by hydrogen bonds between their main chain and side chain amides, to form β sheets and potentially to lead to protein aggregation and precipitation. Circular dichroism, electron microscopic and x-ray diffraction studies of synthetic peptides, and an engineered protein provide *in vitro* evidence supporting the formation of β strands and possibly β sheets by glutamine repeats. Alternatively, it has been suggested that the covalent modification of glutamines by an isopeptide linkage to lysine by the enzyme transglutaminase could also lead to an insoluble precipitate of proteins containing long stretches of glutamine (70).

In support of the polar zipper hypothesis, an *in vitro* filter assay was used to demonstrate that a short truncation

(exon 1 only) of the huntingtin protein with an expanded glutamine repeat can aggregate to form amyloid-like fibrils (71,72). These fibrils showed green birefringence when they were stained with Congo red and viewed by polarized light microscopy, consistent with the presence of β sheets. Aggregation did not occur when the polyglutamine repeat was of normal length. In addition, aggregation only occurred when a huntingtin fragment with an expansion of typical length was first cleaved from the carrier protein to which it was fused during synthesis for the assay. The implication, consistent with cell and mouse models described later, is that generation of a proteolytic fragment of HD may be an important step in HD pathogenesis. In fact, consensus cleavage sites for caspase-3 (also termed apopain or CPP32) exist at approximately position 513 and 530 of huntingtin, and huntingtin can be cleaved by purified caspase-3, caspase-6, and caspase-8.

Cell Models

Research into the pathogenesis of HD has been greatly facilitated by the development of cell models. Two approaches have been used. In the first, huntingtin is introduced into cells through transient transfection; in the other, cell lines are engineered that stably express huntingtin (77–80). In general, short truncations of huntingtin containing the expanded polyglutamine appear to be much more toxic than full-length huntingtin and more liable to aggregation (81–83). However, aggregate formation and cellular toxicity can be disassociated, a finding suggesting that cell toxicity is not related in a simple way to aggregation (79,84,85). Elimination of caspase cleavage sites may reduce the toxicity of mutant huntingtin (9,73–76). Cell death does not correspond to all characteristics of apoptosis, but it can be decreased or blocked in several models with caspase inhibitors (79,84).

A role of nuclear localization for huntingtin toxicity has been suggested, but it is still not proved. Transfection of primary neurons with constructs incorporating a nuclear export signal diminished toxicity, whereas the addition of nuclear localization signals appeared to enhance toxicity (77,79) However, other studies suggested that both the nucleus and the cytoplasm can be the site of pathogenesis (86).

Transgenic Mouse Models

Transgenic animal models have provided some of the most striking evidence for the *gain-of-function hypothesis* of HD pathogenesis. The first animal model of HD was constructed using exon 1 of huntingtin with a very long expanded repeat (87). These animals developed progressive neurologic deficits strikingly similar to those of HD, including incoordination, abnormal involuntary movements, seizures, and weight loss (88,89). However, unlike patients with HD, neuronal cell loss is not prominent. These mice

also developed intranuclear inclusions containing the truncated huntingtin transgene product, but not the endogenous huntingtin protein (48). The intranuclear inclusions are present at the time, and perhaps before, the animals have neurologic signs or brain or body weight loss. The intranuclear inclusions are clearly distinct from the nucleolus, and no membrane separates them from the rest of the nucleus.

Several other HD mouse models have been constructed. A truncated N-terminal fragment of huntingtin driven by the prion protein promoter resulted in mice with features very similar to the initial model (90): the mice develop progressive hypoactivity, incoordination, and weight loss, and on neuropathologic examination they have both intranuclear inclusions and neuritic aggregates. A transgene consisting of a full-length huntingtin cDNA driven by the CMV promoter resulted in a line of mice with a rather different phenotype, characterized by early weight gain and hyperactivity followed later by hypoactivity. These mice have both intranuclear inclusions and some loss of neurons (91).

Perhaps the most promising mouse model of HD involves the use of YAC constructs, so the transgene consists of the entire human HD gene, including the human HD promoter and all introns, with an expanded repeat. These mice develop neurologic signs, electrophysiologic abnormalities, and a shortened life span (92). A single founder with a long repeat had striking evidence of selective striatal neurodegeneration and nuclear localization of N-terminal epitopes of huntingtin in striatal neurons. If additional lines can be generated, this model may be the closest to the human disease of any model yet generated.

Another model of potential utility was generated by inserting an expansion of polyglutamine into the mouse huntingtin gene, thus avoiding the confounding factor of the presence of the human transgene. So far, these mice have not developed neurologic signs, and no neuronal loss has been detected (93). There is evidence of translocation of huntingtin into the nucleus in striatal neurons. Thus, these mice may model early aspects of HD pathogenesis and could provide a useful model for studying the early features of the disease.

The construction of an inducible mouse model of HD has yielded insight into HD pathogenesis (94). A transgene containing exon 1 of huntingtin with an expanded glutamine repeat under the control of the tet-off system was inserted so that the timing of transgene expression could be externally controlled by the presence or absence of an antibiotic in the animals' food. With the transgene on, mice developed neurologic signs and neuropathologic changes including nuclear inclusions. Remarkably, when the expression of huntingtin was turned off, these abnormalities partially reversed. This surprising result suggests that the brain may have more restorative and plastic ability than previously appreciated, and that if the pathologic changes of HD could be halted, substantial repair would perhaps be possible.

Invertebrate Models

Invertebrate models offer the potential of using powerful genetic techniques to search for genetic factors that enhance or suppress an experimentally induced phenotype. Several *Drosophila* models of polyglutamine-induced neurodegeneration have been generated (95–97), with many of the same features of neuronal degeneration observed in mammalian cell models and mouse models. Genetics screens have been used to demonstrate that molecular chaperones such as HDJ1 and HSP70 can suppress the phenotype (97,98). *Caenorhabditis elegans* models may also prove to be of similar value (99,100).

A MODEL OF POLYGLUTAMINE PATHOGENESIS

A model for HD pathogenesis is depicted in Figure 125.5. Several of the steps are speculative, and, as has been evident in this summary, the data supporting the model are at times conflicting. Here we highlight areas of uncertainty:

Evidence for pathogenetically significant proteolytic cleavage of huntingtin remains indirect. The finding that nuclear inclusions can be labeled for N-terminal epitopes, but not for internal or C-terminal epitopes, is consistent with proteolytic cleavage, but it could also result from masking of epitopes. In addition, details of the cleavage, including whether it is processive or endoproteolytic and where in the cell it may occur, are uncertain. The figure shows cleavage taking place in the cytoplasm, but this is speculative.

Another uncertainty is the role of aggregation. It seems likely that the mutant protein adopts an abnormal conformation and that this is a necessary step in pathogenesis. However, the large inclusion bodies visible by light microscopy may represent a downstream event, an epiphenomenon, or even a protective reaction, and therefore they may not be directly tied to pathogenesis. Our hypothesis is that mutant huntingtin adopts an abnormal conformation, leading to abnormal interaction with other proteins, including the sequestration of proteins that contain polyglutamine repeats. An example of such protein is CREB binding protein, an important transcriptional regulator; sequestration of this and similar proteins could have marked effects on neuronal function and survival.

The relative importance of nuclear and cytoplasmic events also remains unclear. In our model, the location of huntingtin in the nucleus leads to altered gene transcription. However, huntingtin could act also within the cytoplasm to interfere with proteins that could otherwise be imported into the nucleus. Furthermore, huntingtin may interact with cytoplasmic molecules, including microtubules or microtubule motors, caspase adaptors, and other proteins, to yield toxicity.

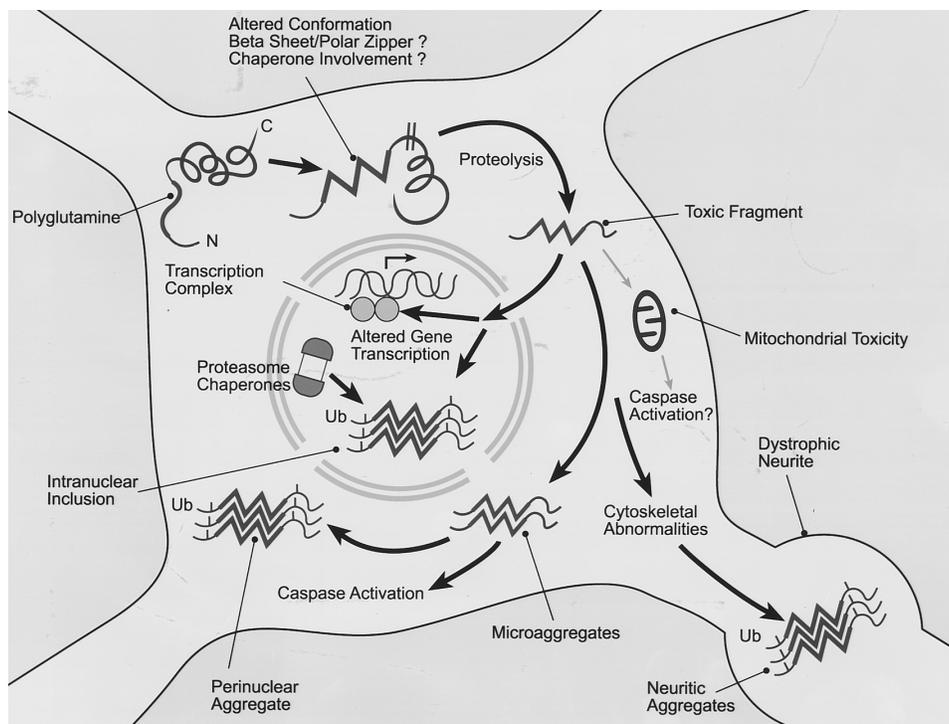


FIGURE 125.5. A model of HD pathogenesis. We propose that HD pathogenesis begins with altered conformation of the protein containing the expanded polyglutamine repeat. Proteolysis generates a fragment that leads to toxicity through several pathways. Nuclear importation may lead to altered gene transcription with a detrimental effect on cell survival. Inclusions also form in the nucleus but may not be a major cause of cell death. Huntingtin fragments may interfere with mitochondrial energy metabolism, either directly, or more likely indirectly, perhaps by altered gene transcription. Microaggregation of the fragment may lead to caspase activation and the consequent initiation of cell death pathways. Fragments may be transported into neurites, interfering with cytoskeletal function. Toxicity may also be mediated by the full-length huntingtin protein with the expanded repeat. As discussed in the text, many of the steps remain speculative.

THERAPY

Recent biochemical, cell, and animal studies are beginning to suggest approaches for development of rational therapeutics (Fig. 125.6). As described earlier, current therapeutics for HD are limited to symptomatic treatments, so any intervention that can stop or slow disease progression would be a major advance.

The first generation of agents designed to slow the progression or delay the onset of HD has emerged from neurotoxicologic models of HD. As of this writing, a major multicenter trial is under way, termed the CARE-HD Study (Coenzyme Q and Remacemide Evaluation in Huntington's Disease). Coenzyme Q is a mitochondrial cofactor, and remacemide is a glutamate-receptor antagonist, and both drugs have efficacy in neurotoxicologic mouse models. The CARE-HD Study is the first multicenter HD drug trial and involves 340 patients treated for 30 months under the sponsorship of the Huntington Study Group. Neurotoxicologic models have also led to smaller trials of other agents, including vitamin E, idebenone, and lamotrigine. None of these agents has demonstrated any clear efficacy (101–105).

Another approach to HD therapeutics involves transplantation either of fetal striatal cells or of cells secreting growth factors. These methods have met with at best limited success so far, but they may have promise for the future. Stem cells, if such cells can be differentiated into striatal neurons in a controlled fashion, may have great potential as therapeutic agents.

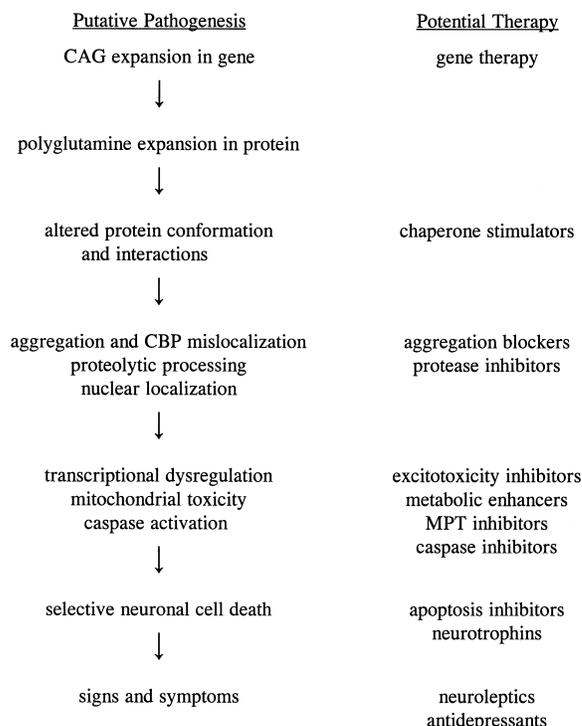


FIGURE 125.6. Approaches to Huntington disease (HD) therapy. The pathogenetic model outlined in Fig. 125.5 suggests certain strategies for slowing, stopping, or even preventing the manifestations of HD.

A screen for therapeutic agents that may alter polyglutamine aggregation is currently in progress using the filter assay developed by Wanker and colleagues. Early results indicate that Congo red and related dyes that intercalate into β sheets (106) and members of the heat shock protein–chaperone family (16) can reduce aggregation. The effect of these agents in animal or cell models of HD is unknown, and even if effective, it is unclear whether these agents would themselves be good candidates for therapeutic compounds. Nonetheless, these results are very encouraging. New compounds may emerge based on those already shown to be effective *in vitro*, or entirely new classes of effective agents may be discovered with further screening.

Mouse models are also in use to screen for therapeutic compounds. Based on work in cells, the role of caspase inhibitors on disease progression has been investigated. Caspase inhibition led to a modest but significant beneficial effect in the exon 1 HD transgenic mouse model (107). A genetic cross of these transgenic mice with a line of mice overexpressing a caspase 1 dominant negative construct (and hence deficient in caspase-1 activity) also suggested that caspase inhibition can slow disease progress. Creatine, chosen based on its effect in neurotoxicologic models of neuronal cell death, also has a significant effect on disease progression in the exon 1 HD transgenic mouse (108). Minocycline, which has shown some efficacy in ischemic models, also has a beneficial effect on this mouse line (109). The magnitude of the effect on disease progression observed in these studies is significant but modest. A similar effect can be induced by altering the environment in which the mice are raised (110). It is possible that a combination of several of these agents may have enhanced effectiveness. Moreover, most of the agents tested so far have been targeted at relatively downstream points in the presumed pathogenetic pathway; agents that are targeted at earlier steps in the pathogenetic pathway may be more effective. As understanding of the pathogenesis of HD advances, the development of therapeutics should follow soon thereafter.

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