Huntington disease (HD) is a neurodegenerative disease that affects about 1 in 10,000 individuals. Symptoms include uncontrollable choreiform or dance-like movements, impairment in memory and reasoning ability, and alterations in personality. The average age of onset of symptoms is about 40 years. The symptoms become increasingly disabling with time, and patients die 15 to 20 years after the first signs appear. Children constitute approximately 10% of HD cases. They are more severely affected than adults and suffer more rigidity in movement.

HD is an autosomal dominant disorder. This means that any individual with one affected parent has a 50% chance of inheriting the gene. The gene that causes HD was identified in 1993 and was initially termed \( IT15 \). The mutation that was discovered within the gene was an unstable expansion of the trinucleotide CAG (please see last month’s column). This trinucleotide encodes the amino acid glutamine and is found within the region of the gene that encodes for protein. As a result, the gene defect in HD causes more glutamines to be placed near the N-terminus of the protein, which is called huntingtin. The normal number of glutamines in huntingtin is between 6 and 34. It expands to 37 or more in the mutated protein. For unknown reasons, there is greater instability and expansion of the CAG repeat region when the gene is transmitted from the father than from the mother.

Fig. 1 The schematic diagram depicts a vulnerable neuron in a brain affected by Huntington disease and shows the variety of changes in mutant huntingtin localization that reflect the altered proteolysis and transport of the mutant protein compared with wild-type huntingtin. In the nucleus, mutant huntingtin accumulates diffusely. The N-terminus is cleaved and forms aggregates or inclusion bodies. In the perinuclear cytoplasm, huntingtin associates with numerous endosomal/lysosomal organelles. The N-terminus of mutant huntingtin also forms inclusion bodies within the dendrites and axons of the cell. These inclusions cause the neurites to become dystrophic. Changes in mutant huntingtin localization may be responsible for the cellular dysfunction and cell death that is the neuropathological hallmark of the illness.
As has been found with other disorders caused by an expansion of triplet repeats, there is an inverse correlation between the degree of expansion and the time of onset of the disease. Thus, large expansions with 55 or more glutamines are associated with onset of symptoms in childhood, while minimal expansions of only 37 to 39 glutamines may result in no symptoms at all. A simple DNA test can now be performed to confirm the presence of the mutation, and genetic counseling is imperative before testing to review the implications of a positive or negative outcome.

The function of the normal huntingtin protein remains unknown. The protein is present in diverse organ tissues, but its highest level of expression is in the brain and the testes. Deletion of the huntingtin gene in mice is lethal in the embryo before the brain is formed. This suggests an important function for the normal huntingtin protein during development. Huntingtin is expressed in the developing brain, rises markedly in the postnatal period in parallel with the differentiation of neurons, and reaches maximum levels in the adult nervous system. Most neurons throughout the brain contain huntingtin, distributed throughout the cell body and into dendrites and axons. Huntingtin exists in a soluble form in the cytoplasm and attaches to microtubules as well as to intracellular and plasma membranes. Based on this localization pattern, huntingtin was suggested to be involved in the transport of vesicles from one compartment of the neuron to another. Huntingtin is sometimes found in the nucleus. Since many proteins that contain a long tract of the amino acid glutamine are known to function in the nucleus as regulators of gene expression, such a role for huntingtin has also been proposed.

It is believed that the mutated protein does not interfere with the function of the normal huntingtin protein, but rather acquires a new property that leads to cell death. This point underscores the fact that mutations in genes do not function only by disrupting the activity of the protein. Sometimes a mutation will lead to a completely new activity of the protein in question. These mutations are called "gain of function" mutations, and this explains why they are autosomal dominant mutations. The presence of a normal copy of the gene will not counteract the deleterious effects of the abnormally active protein.

Individuals carrying the HD gene express mutant huntingtin in neurons throughout the brain, along with normal huntingtin expressed from the normal copy of the gene on the other chromosome. The brain regions that show the most severe loss of neurons in HD are the striatum and the cortex. What causes greater cell death in striatal and cortical cells than in other neurons is an enigma. Striatal cells are known to be particularly sensitive to metabolic stress and to injury by excitatory amino acids. These factors have long been suspected to contribute to HD pathology, but how they are influenced by mutant huntingtin is unclear. Peptides consisting solely of glutamine residues can form insoluble complexes. This led to speculation that mutant huntingtin, and other disease proteins with expanded polyglutamine regions, could aggregate in the brain. Indeed, studies subsequently showed that the mutant huntingtin with an expanded N-terminal polyglutamine region aggregates in the nucleus and in the cytoplasm of the cell. Aggregates also form in axons, which degenerate into what are now called dystrophic neurites.

The aggregated mutant huntingtin colocalizes in complexes with ubiquitin, a protein that is known to assist in the removal of abnormal or misfolded proteins. Ubiquitin is often associated with a protein that is targeted for destruction by the proteasome, a cellular machine that degrades proteins. The occurrence of these complexes, or inclusion bodies, in the nucleus and cytoplasm of the patient's brain is dependent on the length of the polyglutamine tract. When there is a long expansion, as occurs in children with HD, up to 50% of neuronal nuclei in the cortex may have inclusions. In adult cases, only 5% to 7% of neurons in the cortex develop inclusions in the nucleus, while more protein aggregates in axons.

Cultured striatal cells overexpressing mutant huntingtin form inclusions in the nucleus and cytoplasm similar to those seen in the HD brain. These inclusions do not appear to be directly involved in cell death. Treatment of cells with a caspase inhibitor increases the survival of the cells but does not alter the rate of inclusion formation. Conversely, inclusion bodies are reduced by treatment with another caspase inhibitor; however, that treatment does not alter cell survival.

Although nuclear inclusions are not sufficient to cause cell death, the presence of mutant huntingtin in the nucleus may be necessary. This was determined by excluding mutant huntingtin from the nucleus. One can do this experimentally by adding a nuclear export signal of several amino acids to the N-terminus of the protein. When this was done in cultured rat striatal neurons, mutant huntingtin was effectively excluded from the nuclei and cellular death, or apoptosis, was blocked.

Inclusions have been identified in other neurodegenerative diseases such as spinocerebellar atrophy, Machado-Joseph disease, and dentatorubropallidoluysian atrophy. All of these disorders are caused by expansion of a triplet repeat, and a polyglutamine expansion is therefore found in the disease protein. Although symptoms and brain pathology differ in each condition, the formation of inclusion bodies suggests that they share a similar disease process. The inclusions have become a pathological signature of polyglutamine expansion diseases and, for biological research in cells, a useful indicator that the mutant protein has exerted some effect on the cells.

One possibility that is being tested in a number of laboratories is that mutant huntingtin binds to proteins differently than does normal huntingtin and thereby alters cell function. The search for binding partners for huntingtin has uncovered at least 14 potential interactors, but only a few of these proteins have known functions. Many of the proteins identified in this man-
were found to associate with the N-terminal polyglutamine-enriched region of huntingtin. It is interesting that some of the proteins that are known and bind differently to normal and mutant huntingtin have a role in organelle and vesicle transport. Therefore, one possible effect of mutant huntingtin may be to disrupt protein transport from neuronal cell body to both dendrites and axons. Consistent with this idea, studies of HD brains show that there is early pathology in the dendrites of cortical and striatal neurons and degeneration of corticostriatal axons that accumulate N-terminal mutant huntingtin. The diversity of protein partners for huntingtin, including some that localize or function in the nucleus, suggest that multiple functions could be disrupted in neurons in HD.

The expression of polyglutamines in the mouse brain is highly toxic. Mice were genetically engineered to express a large polyglutamine tract inserted into a small protein that is normally innocuous. These mice now develop seizures, motor disturbances, and ubiquitin-positive inclusion bodies. When mice express the N-terminal portion of human huntingtin with a highly expanded glutamine stretch, they develop motor deficits and die within 3 months of birth. These animals show numerous inclusions positive for huntingtin and ubiquitin and a reduced brain size. Despite the severity of this phenotype, however, there is no loss of neurons in the striatum, which is the hallmark of HD pathology.

Mice develop motor deficits more slowly when the polyglutamine expansion is placed in a larger N-terminal fragment of mutant huntingtin or in the full-length mutant huntingtin. Under these conditions, the mice also show cell loss in the striatum. This suggests that the context in which polyglutamine expansions are presented to the cell is important in conferring an HD-like phenotype in the mouse. The reason for the selective loss of striatal neurons in HD, though still elusive, clearly involves characteristics of huntingtin’s function that are unique to these cells.

The successful development of HD mice and cell culture models has helped move scientists into the most exciting and challenging phase of HD research, namely, the search for more effective therapies and eventually a cure.

WEB SITES OF INTEREST
http://www.hdsa.org/
http://www.brc.cam.ac.uk/people/sbd/hdprog.htm

ADDITIONAL READINGS
Kim M, Lee HS, LaForte G et al. (1999), Mutant huntingtin expression in clonal striatal cells: dissociation of inclusion formation and neuronal survival by caspase inhibition. J Neurosci 19:964–973

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To read all the columns in this series, visit the Web site at http://infom.med.yale.edu/childrens/children/index.php.

EXPLAINING RECENT INCREASES IN STUDENTS’ MARIJUANA USE: IMPACTS OF PERCEIVED RISKS AND DISAPPROVAL, 1976 THROUGH 1996, Jerald G. Bachman, PhD, Lloyd D. Johnston, PhD, Patrick M. O’Malley, PhD

Objective: Marijuana use among high school seniors increased during most of the 1970s, decreased throughout the 1980s, and has been increasing again during the 1990s. Earlier analyses of the classes of 1976 through 1986 attributed the historic trends during that period to specific changes in views about marijuana. This study examined whether recent increases in marijuana use among seniors and among students in earlier grades reflect similar processes. Methods: Multivariate regression analyses were conducted on data from large annual nationwide surveys of high school seniors from 1976 through 1996 (approximate n = 61,000) and 8th and 10th graders from 1991 through 1996 (n = 87,911 and 82,475, respectively). Results: Individual lifestyle factors (grades, truancy, religious commitment, evenings out for recreation) correlated substantially with marijuana use but did not explain the historic changes in marijuana use. Rather, decreases in perceived risk of harmfulness and in disapproval can account for the recent increases in all 3 grades and for earlier decreases among seniors. Conclusions: These findings indicate that perceived risks and disapproval are important determinants of marijuana use. Accordingly, prevention efforts should include realistic information about risks and consequences of marijuana use.