Minireview

THE GENETIC DEFECT CAUSING HUNTINGTON'S DISEASE: REPEATED IN OTHER CONTEXTS?

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Huntington's disease (HD) is a dominantly inherited, untreatable neurodegenerative disorder involving progressive chorea, psychiatric changes, and intellectual decline (1). The most characteristic feature of HD is its peculiar movement disorder which begins subtly and progresses to exaggerated dance-like motions that consume the entire body. HD occurs equally in both sexes and is found in all races, but most frequently (~1 in 10,000) in people of Western European descent (2). Although symptoms may begin at any age, they are usually first manifested between the ages of 30 and 55 and they progressively worsen until death 12–18 years later.

The clinical progression of HD is paralleled by neuronal degeneration in the brain. The hallmark of HD is the loss of medium spiny GABAergic projection neurons in a gradient progressing along posteroanterior, dorsoventral, and mediolateral axes of the caudate nucleus (3). Prior to cell death, signs of neuronal dysfunction are evident in recurved dendritic endings and changes in spine density, shape, and size (4). The disorder eventually destroys the architecture of the caudate nucleus and the adjacent putamen, although extensive cell loss also occurs in other regions of the basal ganglia and in the deep layers of the cerebral cortex (5,6). Overall brain weight may be reduced by 25% or more.

Although the proximate cause of the neuronal dysfunction and death is not yet known, it is ultimately due to the presence of a mutant gene located near the telomere of the chromosome 4 short arm (7–9). The HD mutation, discovered in 1993, occurs in the first exon of a 67-exon gene encoding a large novel protein (10–12). All HD patients have an expansion in a sequence of consecutive CAG codons that lengthens the stretch from the 10 to 34 repeat units seen on normal chromosomes to more than 36 repeat units. The major outstanding question since discovery of the HD gene is, how does the expanded CAG repeat cause specific neuronal loss? Delineation of similar CAG expansion mutations in a number of other neurodegenerative disorders suggests that the eventual answer may reveal a common mechanism of neuronal toxicity mediated by the mutant gene products.

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GENOTYPE:PHENOTYPE CORRELATIONS

Once a disease gene has been identified, the pursuit of genotype:phenotype correlations can represent a fruitful approach for gaining insight into pathogenesis. In most disorders, this approach involves comparison of the phenotypic effects of different mutations in the same gene. However, numerous studies have established that the HD CAG repeat expansion is the sole mutation responsible for all bona fide inherited and sporadic cases of HD (13–42). Thus, in HD, the "genotype" is an assessment of the number of CAG repeat units in the person's HD allele, whereas the "phenotype" can represent any of a number of descriptive clinical parameters, such as neuro-

Molecular Medicine, Volume 3, Number 4, April 1997 238-246

logic symptoms, psychiatric symptoms, cognitive symptoms, age at onset, age at death, rate of disease progression, degree of neuropathology, etc.

To date, the relationship between the size of the CAG repeat and the age at onset of neurologic symptoms has been examined in numerous data sets (43). Most HD cases show adult onset and are associated with CAG allele lengths of 40 to 50 units. Disease alleles with CAG expansion in the 35 to 40 unit range may show very late onset or, in some cases, may be nonpenetrant. By contrast, individuals with more than 60 CAG units typically show onset of HD in their juvenile years. All of these studies report an inverse correlation between CAG repeat length and age at onset of neurologic symptoms. However, there is considerable variation in the age at onset associated with any given CAG repeat length (Fig. 1), suggesting that it can be modified by environmental, genetic, or stochastic modifiers. These effects can be minimized by considering the mean age at neurologic onset associated with any repeat length. By combining a number of published data sets (Fig. 1), we have established that mean age at onset does not vary linearly with CAG repeat length, but rather the relationship is best described ($r^2 = .97$) by an exponential model:

$$y = 284.49e^{-.043x}$$

where y is the age at onset and x is the number of CAG repeat units.

This model is consistent with the view that during the lifetime of the HD individual, there is a progressive decline in the function of the striatum, due to neuronal dysfunction or cell death, that leads to onset of symptoms when a critical threshold of functional loss is reached. The rate of striatal decay in this scenario would be an exponential function of CAG repeat length and one would expect that some degree of neuronal dysfunction or cell loss would precede neurologic manifestations. Extrapolation of neuronal cell counts reported from postmortem HD brains (44) suggests that a threshold at which neurologic onset occurs corresponds to approximately 30% neuronal loss in the caudate nucleus. Validation of this model will require careful analysis of postmortem HD brains from individuals who expire prior to neurologic onset. The alternative to the gradual neuronal decline model is that the length of the CAG repeat determines, in an exponential manner, the age at which the pathogenic process is first triggered with no prior evidence of dysfunction.

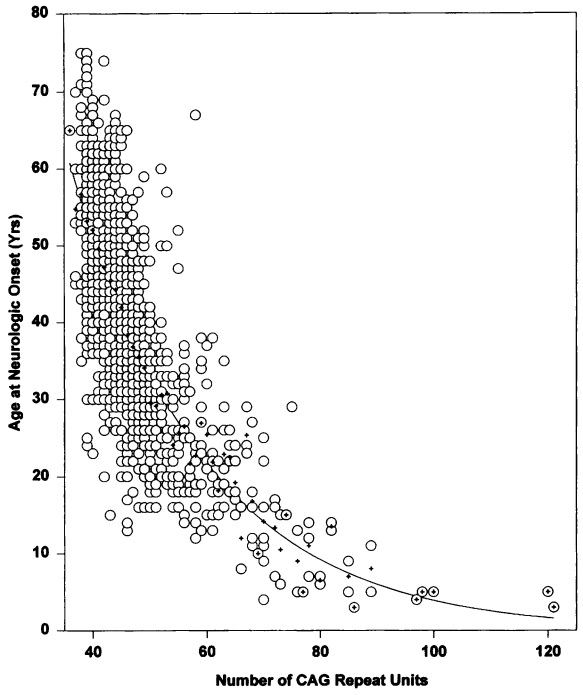

FIG. 1. Relationship between age at neurologic onset and number of HD CAG repeat units

Data compiled from published reports of age at onset (open circles) involving 1226 HD patients (10,13,21,23–26,28–31,34,36,37,42,75–77) permitted the determination of mean age at neurologic onset (+) associated with different numbers of CAG repeats in the disease allele. The solid line represents that predicted by nonlinear regression analysis for the mean age at onset ($r^2 = .97$) using the exponential model presented in the text.

Not surprisingly, CAG repeat length has also been inversely correlated with the age of onset of psychiatric manifestations in HD (13). A similar relationship with age at death (Fig. 2) indicates that the presence of an HD-length CAG repeat produces a reduced lifespan (13). However, despite an apparent increased rate of neuronal loss with increasing CAG repeat lengths, the length of time from onset of neurologic symptoms to death does not show a corresponding relationship. Indeed, the duration of disease from neurologic onset to death is remarkably similar (~15 years) for different repeat lengths (Fig. 2). Thus, progression to death is not highly correlated with the size of the mutant allele, as is neurologic onset, and it probably involves additional factors beyond degree of neuronal loss. Indeed, direct attempts to relate the patient's overall functional

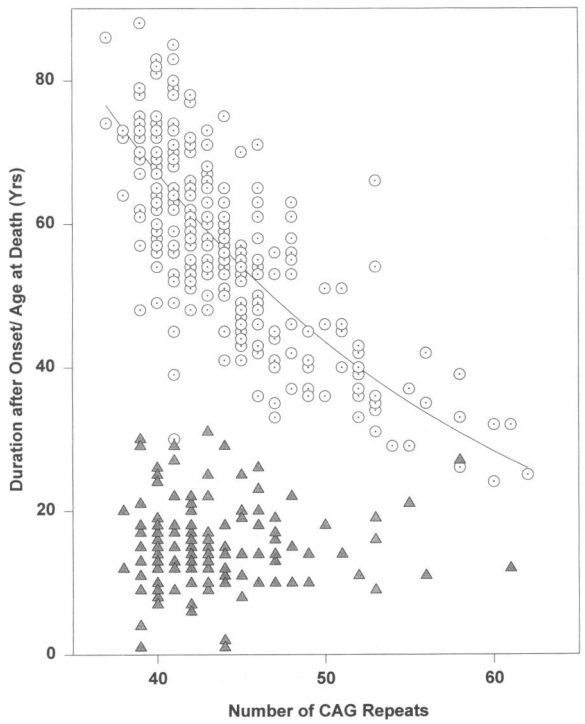

FIG. 2. Duration of disease after onset and age at death versus number of HD CAG repeat units

Age at death (dotted circles) is plotted against the number of CAG repeats for 305 patients whose brains were subjected to postmortem analysis to confirm HD neuropathology (13). The line shows the predicted curve-fit (r^2 = .49) based on a similar exponential model to that used Fig. 1. Also shown is the number of years between onset and death (shaded triangles) for 144 patients whose age at onset and death were both known. Linear regression analysis failed to identify any relationship between the disease duration and number of CAG repeat units.

decline with CAG repeat length have yielded conflicting results (29,30,45).

HD GENE PRODUCTS

The mutant HD gene directs the synthesis of RNA with an expanded CAG segment and consequently a protein with a lengthened stretch of consecutive glutamine residues. Although it is commonly assumed that the dominant mutation acts through the altered protein, both major products differ significantly between normal and disease alleles and therefore the pathogenic effect could equally be due to the altered mRNA. The HD mRNA consists of two alternatively polyadenylated species of 13.5 kb and 10.5 kb, with the CAG repeat located near the 5′ end, 17 codons downstream from the initiator AUG (10). The mRNA encodes an ~350 kD protein, named huntingtin, with no similarity to other reported sequences except in the low-sequence complexity polyglutamine-polyproline region (encoded by the CAG repeat and an adjacent degenerate CCG repeat) near the NH_2-terminus and a motif of unknown function dubbed "HEAT" that has been found in database searches in a variety of unrelated proteins (10,46). Whereas the first 17 amino acids of huntingtin and the remainder of the protein downstream of the polyglutamine-polyproline segment are highly conserved in evolution, the subsequent polyglutamine-polyproline segment is not, and it may be largely dispensable for huntingtin's unknown normal function (47–50). The disease mechanism could operate as a "gain-of-function" that confers a novel, deleterious property on either the HD mRNA or huntingtin protein without simultaneously interfering with their normal physiological roles.

COMPARISON WITH OTHER CAG TRINUCLEOTIDE REPEAT DISORDERS

The mutational mechanism in HD, an expanded, meiotically unstable CAG repeat encoding polyglutamine, has been demonstrated in several other genes causing neurodegenerative disorders, which suggests that a common pathogenic mechanism is triggered in each case (51). Kennedy's disease, or spinal bulbar muscular atrophy (SBMA), is caused by an expanded CAG repeat in the X-linked androgen receptor gene producing, in males, a progressive loss of anterior horn cells in the spinal cord with consequent progressive muscular weakness (52,53). Dentatorubral-pallidoluysian atrophy (DRPLA) involves neuronal loss in the dentatofugal and pallidofugal systems, and consequent ataxia and choreoathetosis, due to an expanded CAG repeat in a chromosome 12p gene encoding atrophin, another protein of unknown function (54–58). Several spinocerebellar ataxias are caused by an expanded CAG repeat, including spinocerebellar ataxias 1, 2, 3, 6, and probably 7.

In SCA1, an expanded CAG repeat on chromosome 6p alters the coding sequence of ataxin-1 (a protein of unknown function) and causes progressive neuronal loss in the cerebellum, the inferior olive, and in various cranial

nerve nuclei (59). A similar disorder, SCA2, in which the most affected regions are cerebellum, pontine nuclei, inferior olives, and substantia nigra, is caused by an expanded CAG repeat in a novel gene at 12q24.1 (60–62). In SCA3, allelic with Machado-Joseph disease (MJD), an expanded CAG segment in a novel chromosome 14q32.1 gene causes progressive degeneration of the spinocerebellar tracts, with relative sparing of the inferior olive and cerebellar cortex compared with SCA1 and SCA2 (63–70). A CAG repeat in the α_{1A} subunit of a brain voltage-gated calcium channel (*CACNL1A4*) at 19p13 has recently been implicated as the cause of SCA6, another dominant cerebellar ataxia, although the repeat is more stable and the expanded polyglutamine stretch is shorter than in the other CAG neurodegenerative disorders (71). The genetically distinct SCA7, causing spinocerebellar ataxia with retinal degeneration, also shows evidence of expanded CAG in genomic DNA and of lengthened polyglutamine in a 130-kD nuclear protein, but the locus has not yet been cloned (72,73).

As Fig. 3 shows for several of these disorders, a relationship exists between CAG repeat length and age at onset that is comparable to the pattern seen in HD. The age at onset data for SCA1 superimpose on the HD curve whereas data for DRPLA and SCA3/MJD are displaced to the right and those for SCA2 are displaced to the left. These findings indicate that in each disorder the degree of CAG expansion necessary to cause a detectable neurologic deficit differs. The slopes of the latter three curves also differ from HD and SCA1, indicating a greater effect of each additional CAG unit on reducing age at onset. These comparisons suggest that in each disorder, the deleterious effects of the expanded CAG act in a similar manner but on target cell populations and with a CAG length dependence that are dictated by the context in which the mutation is expressed. If the model of gradual neuronal decline to a symptomatic onset threshold is correct, individual differences in the curves would reflect corresponding differences in the threshold for different neuronal cell types and for the context-dependent effect of each embedded CAG repeat on the rate of neuronal decline. In the more severe young-onset cases, the neuronal pathology extends beyond the regions most characteristic of each disorder to parts of the brain that degenerate in one or more of the other CAG repeat disorders. This suggests that each CAG repeat might produce progressive neuronal loss

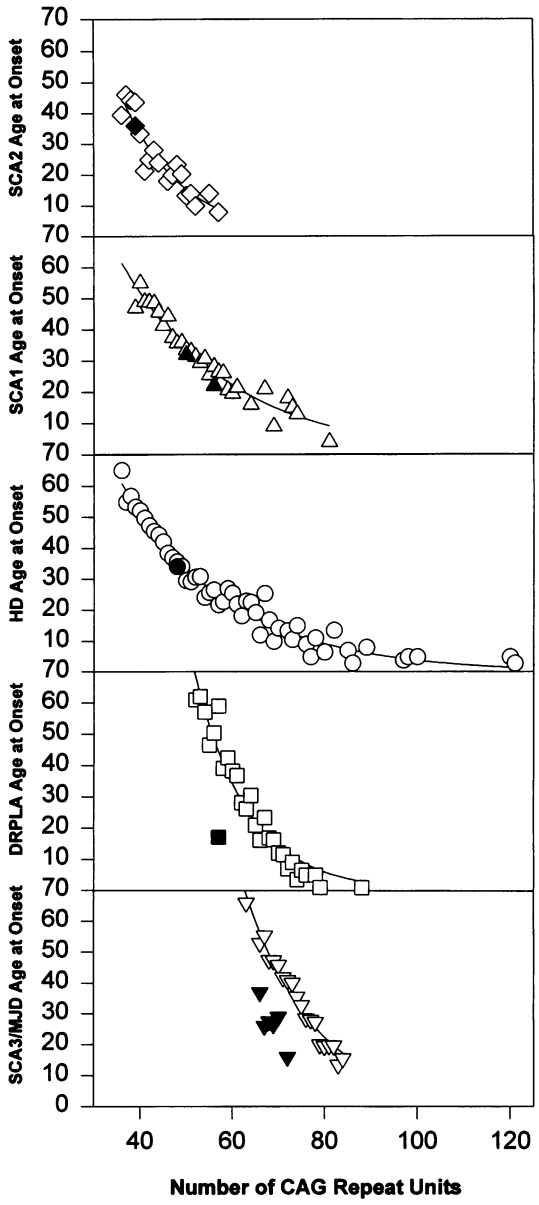

FIG. 3. Onset and repeat length in CAG repeat disorders

Data compiled from published reports were used to calculate mean age at neurologic onset (as in Fig. 1) associated with different CAG repeat lengths in the disease alleles of 92 SCA2 cases (diamonds), 201 SCA1 cases (inverted triangles) (78–81), 1226 HD cases (circles) (10,13,21,23–26,28–31,34,36,37,42, 75–77), 149 DRPLA cases (squares) (58,82–84) and 332 MJD/SCA3 cases (triangles) (64,68,69,85–87). Each panel also shows the curve-fit (line; r^2 = .867 (SCA2); .952 (SCA1); .970 (HD); .943 (DRPLA); .968 (MJD/SCA3) for the mean age at onset with an exponential model similar to that used in Fig. 1. Filled symbols in each panel represent individuals homozygous for the corresponding disease allele, with age at onset plotted against the number of CAG repeats in larger of the two disease alleles (61,81,88–92).

of more than one neuronal cell population, but with a different rate of decline in each population.

A notable feature of these disorders is the age-at-onset phenotype for individuals homozygous for the disease allele (Fig. 3). In HD, SCA1, and SCA2, a second dose of the disease allele does not aggravate the disease process, as the age at onset observed matches well that expected on the basis of the disease allele with the larger repeat. By contrast, in both DRPLA and SCA3/MJD individuals, the combined effects of two disease alleles produces an age of onset earlier than that predicted based on either allele alone. The lack of a significant effect of a second disease allele in HD, SCA1, and SCA2, suggests that the presence of a single mutant gene in these disorders produces sufficient product to maximize the rate of neuronal loss that can be caused by a given length of CAG repeat. On the other hand, in DRPLA and MJD/SCA3, the pathogenic triggering mechanism is not saturated in typical heterozygotes as some capacity to accelerate the process with a second disease allele remains.

MECHANISM OF PATHOGENESIS

Although it is conceivable that the biochemical mechanisms producing neuronal loss in the CAG repeat disorders could differ in each case, the remarkable similarities argue strongly that the pathogenic mechanisms in HD and in the other CAG repeat disorders are closely related. Consequently, defining the pathogenic mechanism in any one of the disorders will probably lead to an understanding of the others and to a delineation of the factors that determine the surprising specificity of neuronal loss. In each case, cell death could either occur as an effect of the long CAG segment in the disease gene mRNA or be due to an expanded polyglutamine segment in the corresponding protein. As each of the genes is unrelated to the others, loss of normal protein function is probably not the primary cause of pathogenesis, although it could certainly contribute to aspects of the disease phenotype (74). Differences in the capacity of the altered gene products in each disorder to trigger the disease process may depend on many factors including their concentration, localization, normal function, and constraints imposed by product structure outside the CAG/polyglutamine region. Hopefully, the ability to compare specific neuronal loss in a number of disorders will accelerate recognition of the steps in pathogenesis that are shared between them, providing the knowledge to develop rational treatments.

ACKNOWLEDGMENTS

The authors' work has been supported by NIH grants NS16367 and NS32765 and by grants from Bristol-Myers Squibb Inc., the Hereditary Disease Foundation, the Huntington's Disease Society of America, and the Foundation for the Care and Cure of Huntington's Disease (FCCHD). This paper is dedicated to the memory of Mr. Dennis Shea, the FCCHDs founder, in recognition of his many critical contributions to the research effort directed at finding a cure for HD.

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