Mutation size and age at onset in Huntington's disease

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Abstract

The mutation responsible for Huntington's disease is a polymorphic (CAG)_n repeat sequence which is expanded on affected chromosomes. The number of repeat units observed on 229 affected chromosomes varied from 27 to 102, while the control chromosomes showed a range of 7 to 34 repeats. There was a highly significant relationship between the size of the expanded region and age at onset, larger mutations being associated with earlier onset. This association was strongest in those with onset before 25 years of age but less clear cut with later onset, and is therefore unlikely to be useful for predicting age at onset in the context of presymptomatic testing.

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Huntington's disease (HD) is a chronic degenerative disorder of the central nervous system which is inherited as an autosomal dominant trait. Affected subjects suffer from chorea, impaired motor coordination, dementia, and a variety of psychiatric problems; the disorder follows a progressive course leading eventually to death after an interval of about 15 to 20 years.¹ The onset most commonly occurs between the ages of 30 and 50 but can vary from early childhood to the seventh or eighth decade, and the clinical manifestations also show a good deal of variability with a tendency to more rapid progression and atypical neurological features in those with an earlier age at onset.

The gene responsible for HD was localised several years ago by linkage analysis to chromosome 4, and more recently a newly identified gene of unknown function (IT15) within the relevant candidate region has been shown to contain a (CAG)_n repeat sequence which is expanded and unstable in affected subjects but not in normal controls.² This is similar to the mechanism recently identified as the cause of both myotonic dystrophy³ and fragile X mental retardation.⁴ An interesting feature of the reported HD mutation was the suggestion of an inverse relationship between the size of the expanded region and the age at onset of the disease (a phenomenon also seen in myotonic dystrophy) and this initial observation has been confirmed in subsequent reports.⁵⁻⁷

The discovery of the mutation responsible for HD has important implications for both research and clinical practice. The ability to test for the presence of the mutation in apparently affected subjects without a family history of HD, and in at risk subjects who lack the appropriate family structure for predictive

testing by genetic linkage, has created an urgent need to establish the range of mutation sizes in affected and normal subjects before the new test can be introduced, while the observation that age at onset may be dependent on mutation size has obvious implications for predictive testing. We report here the results of testing for the number of (CAG)_n repeats in affected subjects and their spouses known to the North Western Regional Genetic Register for HD families.

Methods

PATIENTS

DNA was available from 234 affected subjects and 86 spouses known to the Regional Genetic Register service. Most of the affected persons had been examined personally by a member of the Genetic Register team, but a few samples had been obtained through other clinicians from affected family members in distant parts of the country. Samples from at risk subjects were not examined, apart from a small number who had previously received adverse predictive test results, where DNA was also available from the affected parent for comparison. These at risk samples were not included in the main analysis of mutation size in affected persons.

Data regarding age at onset was obtained from the clinical case notes, based on the first appearance of unequivocal clinical features of HD. In view of the obvious difficulty of establishing a precise point in time for the onset of clinical disease, patients were assigned to one of four age at onset categories: <25 years, 25 to 40 years, 40 to 65 years, and >65 years.

LABORATORY METHODS

Non-isotopic amplification of the (CAG)_n repeat was carried out using primers modified from the original report by the HD Collaborative Group.² The products were separated on 6% denaturing polyacrylamide gels and visualised using the silver staining method developed in our own laboratory. Fixed gels were made permanent by drying between two sheets of moistened cellophane and photographed. Allele sizes were estimated relative to a log plot of ϕ X174 DNA sequence markers.

Results

Results were obtained from 234 affected subjects, but for six cases the number of repeats seen in the larger allele fell well within the range seen in normal persons. One of these patients proved to have a spouse with an ab-

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Table 1 Number of repeats on normal chromosomes.

	Affected subjects	Controls (partners)	Total
Mean	19.63	18.60	19.19
SD	3.50	3.80	3.67
Min	11	7	7
Max	34	34	34
No	229	172	401

Table 2 Number of repeats on HD chromosomes.

	Male	Female	Total
Mean	45.60	46.91	46.34
SD	4.63	8.99	7.42
Min	39	27	27
Max	64	102	102
No	100	129	229

normal result, and it was assumed that the two samples had been confused; of the remaining five, four were isolated cases of presumed HD and the other was a person who had not been personally examined in this department. These five samples were therefore excluded from further analysis.

The number of (CAG)_n repeats detected on 401 normal chromosomes (both alleles from 86 controls and the smaller allele from 229 affected subjects) and 229 affected chromosomes are summarised in tables 1 and 2 and displayed graphically in fig 1. No significant differences in the number of repeats were detected between the affected chromosomes of male or female patients; however, the mean number of repeats was significantly greater for those of paternal than maternal origin (*t* test, *p* < 0.001) (table 3).

The relationship between mutation size and age at onset in affected subjects is shown in tables 4 and 5. The mean number of repeats in the larger allele (table 4) was greatest in those with an onset before 25 years of age, and least in those with a very late onset after 65 years of age. The two subjects in our series with an onset before the age of 10 years accounted for the highest values of 102 and 86 repeats, respectively. This inverse relationship between the size of the larger allele and age at onset was

Table 3 Number of repeats on HD chromosomes.

	Maternal origin	Paternal origin
Mean	45.46	49.99
SD	4.95	10.36
No	96	67

(*p* < 0.001).

Table 4 Number of repeats by age at onset.

	Age at onset				Total
	< 25 y	25–40 y	40–65 y	> 65 y	
Mean	61.35	47.56	43.26	41.17	46.60
SD	12.78	4.166	2.69	1.17	7.64
95% CI for mean	55.82–66.87	46.55–48.58	42.76–43.76	39.94–42.39	45.56–47.65
Min	46	38	27	40	27
Max	102	64	50	43	102
No	23	67	111	6	207

(*p* < 0.001).

highly significant (*p* < 0.001), with no overlap between the 95% confidence intervals for the mean number of repeats. No such relationship could be shown between age at onset and the size of the normal allele in these patients (table 5).

In view of the previous observation that paternal transmission of the HD mutation is associated with a tendency to earlier onset in the child, we examined the correlation between the number of repeats in the mutant allele in affected parent-child pairs. Data were available from a total of 21 pairs; in 11 cases the mother was the affected parent, and in the remaining 10 it was the father who was affected. It should be noted that 11 of these pairs involved examination of DNA from a child who had had an adverse predictive test result but is not yet symptomatic. The mean number of repeats between parent and child was highly correlated regardless of the sex of the transmitting parent (table 6). However, the mean number of repeats in offspring of affected fathers was greater by 7.44 than the mean for the fathers, while no such increase was noted in the children of affected mothers.

Figure 1 Number of repeats on normal and HD chromosomes.

Table 5 Size of normal allele by age at onset.

	Age at onset				Total
	< 25 y	25–40 y	40–65 y	> 65 y	
<i>Maternal</i> (Paternal mutation)					
Mean	18.36	19.30	19.20	20.00	19.07
SD	4.41	3.15	2.616		3.265
95% CI	15.81	18.05	17.98		18.24
for mean	20.91	20.54	20.42		19.89
No	14	27	20	1	62
<i>Paternal</i> (Maternal mutation)					
Mean	23.00	20.20	19.98	21.50	20.28
SD	5.80	3.76	3.66	0.71	3.84
95% CI	16.92	18.80	18.98	15.15	19.49
for mean	29.08	21.61	20.98	27.85	21.08
No	6	30	54	2	92

Discussion

The results of this study support the previous finding that the (CAG)_n trinucleotide repeat sequence in IT15 is expanded on chromosomes bearing the HD mutation. Of 234 affected subjects examined, only five had a result which fell unequivocally into the range observed in normal subjects and all of these were atypical cases where there was reason to doubt the accuracy of the diagnosis. Results for the remaining 229 ranged from 27 to 102 repeats, while the number of repeats on normal chromosomes varied between 7 and 34. However, the affected subject with 27 repeats was also an isolated case with no family history and a somewhat atypical clinical picture; if this case is also excluded, the results range from 7 to 34 on normal chromosomes and 36 to 102 on HD chromosomes, with no overlap between the two ranges.

The observation that there is no overlap

between the range of results for normal and HD chromosomes has important implications for presymptomatic testing; however, there was very little difference in our series between the largest number of repeats on normal chromosomes (34 repeats, observed on two of 401 chromosomes) and the smallest result on HD chromosomes (36 repeats, observed in three cases). Given the likelihood that results may be inaccurate by up to two or three repeats, it is clearly going to be difficult to interpret results within the 30 to 40 range. The discovery that DNA samples from a husband and wife had been switched at some point in the process from obtaining blood to testing the DNA also underlines the importance of obtaining second samples to guard against errors of this type, since these are less likely to be detected than when predictive tests were carried out using linked markers, involving the need for examination of samples from many other family members.

Our findings regarding mutation size and age at onset also support the initial observation by the HD Collaborative Research Group² of an inverse relationship between the number of repeats and the age at onset of the disease. The largest expansions of the trinucleotide repeat sequence were observed in patients with the earliest clinical onsets, with a highly significant difference between the means for the different age at onset groups. This difference was most marked for those with very early onset, but was observed right across the range of age at onset with no overlap at all between the 95% confidence limits for the mean number of repeats in any of the age groups examined.

Previous studies have shown a relationship between the sex of the affected parent and age at onset in the child, paternal transmission being associated with slightly earlier onset; juvenile onset in particular usually occurs in the context of paternal transmission.⁸ There has been much speculation about possible reasons for this paternal transmission effect,⁹ but the results of the present study indicate a highly significant difference in the number of repeats on HD chromosomes of maternal and paternal origin and there can be little doubt that it is this variability in the mutant HD allele rather than an effect of imprinting or modifying genes elsewhere which is responsible for the phenomenon.

The explanation for this difference between HD chromosomes of paternal and maternal origin can be found in the analysis of affected parent-child pairs summarised in table 6. As would be expected, there were strong correlations between the number of repeats in parent and child, regardless of the sex of the affected parent. However, the mean number of repeats had increased between generations in the offspring of affected fathers, whereas this was not seen in the children of affected mothers. Examination of the individual cases indicates that almost all of this increase was contributed by just three pairs, two of which were associated with onset before the age of 20 years in the affected child. It therefore appears that paternal transmission is associated with the poten-

Table 6 Number of repeats: parent-child correlations.

	Mother affected	Father affected	All pairs
Mean (parent)	46.09	45.80	45.95
SD	3.08	3.85	3.38
Mean (child)	45.36	52.10	48.57
SD	3.61	14.72	10.76
Mean change	-1.0	7.44	2.62
Correlation	0.742	0.899	0.730
p value	<0.01	<0.001	<0.001
No	11	10	21

Figure 2 Age at onset as a function of mutation size.

tial for instability of the mutation at meiosis, accounting for cases of anticipation in the male line.

The finding of an association between mutation size and age at onset raises the question of whether it will be possible to predict the age at onset for presymptomatic subjects receiving an adverse predictive test result. It will be seen from an examination of fig 2 that there were no subjects with fewer than 40 repeats who had an onset before the age of 25 years, and none with more than 60 repeats who were still asymptomatic at 40 years. The majority of subjects have between 40 and 49 repeats, and of these around 31% had an onset before the age of 40, while 69% had a later onset. For those with 50 to 59 repeats, all but 3.7% were affected before 40 years, with 33% having an onset before the age of 25. These observations are likely to be of limited utility in practice because most at risk subjects seeking predictive tests are already over the age of 25 at the time of testing.

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