Huntington's disease in Grampian region: correlation of the CAG repeat number and the age of onset of the disease

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Abstract

The identification of an unstable trinucleotide repeat as the mutation responsible for Huntington's disease (HD) has given the hope that additional information can be provided about age of onset and mode of action of the mutated gene. We present in this paper results of a clinical and molecular study of 82 patients affected with HD from 46 pedigrees within the Grampian region, Scotland. Our results show a correlation between age of onset and size of the CAG expansion. This study has produced no overlap in mutation size between affected and unaffected alleles. The sex of the parent transmitting the mutated allele and the size of the normal allele have no significant effect on the clinical features of the disease. In the three juvenile cases the affected parent was the father but the number of cases is too small to produce statistical significance. An increase in the CAG repeat size is shown in the transmission of the gene in five cases, accompanied by an earlier age of onset in four; in three of these cases, the affected parent was the father. Eleven sib pairs were studied and there is a negative correlation between the difference in age at onset and the difference in repeat size. Thus there is some evidence of a relationship, but this is not statistically significant because of the small numbers involved. The presence of the same or different normal allele had no effect on age of onset in this small group. We suggest that additional factors, as yet unrecognised, influence the age of onset and clinical presentation of HD.

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HD is a neurodegenerative, adult onset disorder with an accepted prevalence of 5-7/100 000.¹ The prevalence in Grampian region is recognised as being unusually high² and is 12/100 000; complete ascertainment of the families in the region has been accomplished. The clinical aspects of the disease include choreiform movements, psychiatric disease,³ and cognitive impairment; these features of HD have variable ages of onset and severity, even within families.

The locus of the gene for HD was described in 1983,⁴ and predictive testing using linked genetic markers has been available for over five years in centres in the UK⁵⁻⁷ and other centres throughout the world. Recombination at this

locus meant that these results were never completely accurate, and it was not possible to predict the age of onset for those who received a high risk result. The identification of the mutation in the gene as a trinucleotide repeat⁸ led to the hope that such additional information could be provided for persons at risk of HD, although severity and age of onset does not appear to correlate closely with trinucleotide repeat number in other disorders with this type of mutation.⁹

The well documented HD families² from the stable population in Grampian region have been studied for over 30 years, and data about age of onset and the clinical features of the disease have been recorded. The uptake of presymptomatic predictive testing has been high,⁷ with 77 results produced in the last five years. The high risk group are regularly reviewed and in some cases the first definite signs of the disorder have been noted. We have studied the size of the CAG repeat in our affected population, and the size of the normal allele, to examine the effect of these two values on the manifestation of the disease in our families. These results are unique to this paper, although 38 of the patients contribute to the review of the CAG repeat range in the affected population in Scotland (Barron *et al*, this issue).

Methods

The expansion of the CAG repeat in DNA from leucocytes was amplified using the PCR method of Warner *et al*.¹⁰ Accurate sizing was determined using radiolabelled dTTP which gives single strand labelling. Our sizes were verified against sequenced clone L191F1. Only DNA samples from Grampian pedigrees were analysed; no samples from persons at risk or of uncertain clinical diagnosis were used.

Results

CAG REPEAT LENGTH

The range of CAG repeat length in our group from 46 kindreds is 39 to 67 and the frequency of the repeat sizes is shown in fig 1. Fig 2 illustrates the range of the normal alleles which is 13 to 33. No difference was noted between the sexes, and there is no overlap between the sizes of the normal allele and the affected allele.

CAG REPEAT LENGTH AND AGE OF ONSET

Fig 3 shows the age of onset and the associated mutation. Our results show a significant nega-

Figure 1 The range of the CAG expansion in the mutated gene.

Figure 2 The range of the CAG expansion in the normal allele.

Figure 3 Age at onset and CAG repeat number.

tive correlation of -0.58 ($p = <0.01$) between the mutation size and age of onset. The three cases of juvenile onset (onset between the ages of 12 and 20 years) were accompanied by expansions of >64 CAG repeats, and were not included in this statistical analysis.

An expansion of >50 is associated with adult onset (onset after the age of 20) in nine of our cases. No affected subject has an expansion less than 39 in our group, which includes 12 cases where onset was later than 55 years.

THE CAG REPEAT SIZE IN SIBS

Table 1 describes 11 sib pairs from the Grampian families. We have found a negative correlation (-0.29) between the difference in age of onset and the difference in size of the CAG repeat, but this was not significant because of the small numbers involved. The presence of the same or different normal allele in the sibs has no effect on age of onset in our group.

A further two sets of sibships of three are not described in table 1. In one trio, two who shared the same normal allele (16), had onset between 39 and 40 years, but their mutation sizes were 46 and 43. The third sib, with a different normal allele (17), but the same smaller mutation size of 43, had onset at 46 to 48 years. The second trio had two sibs with the same CAG repeat sizes (42 in the mutated gene and 22 in the normal allele) and psychiatric mode of onset, but a difference in age of onset of 10 years (29 to 31, 41 to 43). The third sib from this group had a motor onset of disease at the age of 39 to 41, and a mutation size which had increased to 46. In addition the normal allele was smaller (16 CAG repeats). Both sets of sibs had inherited the disease from their mother.

TRANSMISSION OF THE MUTATED GENE

Six parent-offspring results are available (table 2). The transmission of HD was from the mother in three cases and from the father in three, although two sibs are included who inherited the disease from their father. Subjects who have inherited the disease from their father have a greater increase in number of CAG repeats and show an earlier age of onset than those who have inherited the HD gene from their mother. This small number of family groups is not suitable for statistical analysis.

Discussion

The definition of the mutation responsible for HD was long awaited⁴ and there were hopes that it would explain the variety of clinical signs among sufferers, the wide variation in ages of onset, and the phenomenon of anticipation when the disease is inherited from the father.

Our results support the finding that an expansion in the CAG sequence in the HD gene is present in those affected by the disease. Furthermore, we have no overlap in the number of repeats between affected and non-

Table 1 Sibs, change in CAG repeat number and age at onset.

Sib pair: difference in years in age of onset	No of pairs with same non-HD chromosome		No of pairs with different non-HD chromosome	
	Expans same	(Expans diff)	Expans same	(Expans diff)
None	1			
<2			2	
3-4		1* (+2)		1* (+18)
5-10		1* (+1)		1* (+3)
				1 (+2)
				1* (+11)
>20		1 (+1)		1 (+2)

* Father affected.

Table 2 The increase in CAG repeat number on transmission, difference in age at onset, and the sex of the transmitting parent.

Mutation in father (age of onset)	Mutation in mother (age of onset)	Mutation in offspring (age of onset)	Repeat number difference
45 (39-41)		67 (18-20)	+22
39 (49-51)		45* (24-26)	+6
39 (49-51)		43* (27-29)	+4
	43 (29-31)	45 (37-39)	+2
	43 (39-41)	45 (29-31)	+2
	41 (59-61)	41 (42-44)	0

* Sibs.

mal alleles. This confirms the work from other areas in Scotland (Barron *et al*, this issue), although a smaller affected allele size is present in some of the affected population in that study.

We have found a significant negative correlation between age of onset and size of the mutation ($p=0.01$). Earlier work has shown that the affected population in Grampian are more elderly, with a later age of onset, than the average HD population.¹¹ Only about 34% of the variance in onset age can be accounted for by the regression of onset age on mutation size. We have noted larger CAG repeat sizes in the group studied, and this suggests that there are other factors involved in controlling the age of onset of this disorder.

Many of the large, stable Grampian families have documented family histories which can be traced to early in the 18th century. No new mutation has been documented in this region. The relatively large number of CAG repeats in the HD genes in Grampian⁸ may reflect the age of the gene in the families described.

We have found a negative correlation (-0.29) between the difference in age of onset and the difference in size of the CAG repeat in sibs. In the sibs studied, the inheritance of the same or different non-HD chromosome would not appear to affect the relationship between age of onset and the size of the expansion in the gene. The numbers involved mean that these results are not statistically significant, but further work is being undertaken on this group.

The results to date on transmission of the gene are numerically too small for statistical analysis, but it can be seen from table 2 that there is a trend to increase in size of the mutation upon transmission, particularly when through the father. Excluding the three juvenile cases (who inherited the disease from their father) we found no significant effect of the sex of the transmitting parent on the age of

onset of the disease or the size of the mutation found in the patient, although an effect of paternal transmission on age of onset is an accepted phenomenon.¹² Six results from two generations were studied from our group, and these are shown in table 2. Although the numbers are too small for statistical significance, this illustrates the larger expansion in the paternally transmitted allele which was in each case accompanied by an earlier age of onset of more than 20 years. The two sibs described had onset of symptoms more than 20 years earlier than their father, although the mutation has increased only by four and six repeats from 39 CAG repeats. The same size of normal allele was present in both offspring. Where inheritance was maternal, two cases had earlier onset and one later (about eight years), and CAG repeat number was unchanged or slightly increased. We anticipate additional results from subjects from our high risk group to investigate this phenomenon further.

The effect of the normal allele has been considered by Farrer *et al*¹³ who suggested that the expression of HD is modulated by the normal HD allele or by a closely linked locus. This work was accomplished before the definition of the gene. Individual cases from our study are consistent with this hypothesis. One of our patients inherited an unchanged mutation of 41 CAG repeats from their mother but their age of onset is 42 to 44, more than 15 years earlier than their parent. The paternal (normal) allele was 31 in the mother and 23 in her offspring. However, our findings to date show no correlation between normal allele size and age of onset, or with the sex of the unaffected parent and age of onset of HD in the offspring.

These results from the well defined population in Grampian region show that there is a correlation between age of onset and CAG expansion size in the mutated HD gene, but there are other factors which have yet to be defined which are modifying the action of this mutated gene. There is no evidence from our results that the CAG repeat in the normal allele is contributory.

Persons at risk of HD may receive an accurate prediction of their status, but caution is advised about prediction of age at onset or type of onset in this patient group.

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