Reduced penetrance of the Huntington’s disease mutation

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Controversy persists concerning the significance of Huntington disease (HD) alleles in the 36–39 repeat range. Although some clinically affected persons have been documented with repeats in this range, elderly unaffected individuals have also been reported. We examined 10 paternal transmissions of HD alleles of 37–39 repeats in collateral branches of families with de novo HD. All 10 descendants, including many who are elderly, are without symptoms of HD. Forty percent of the transmissions were unstable, although none varied by more than one repeat. The observation that individuals with alleles of 37–39 repeats may survive unaffected beyond common life expectancy supports the presence of reduced penetrance for HD among some persons with repeat sizes which overlap the clinical range. Non-penetrance may be increased in the collateral branches of de novo mutation families when compared to penetrance estimates from patient series. There was no CAA→CAG mutation for the penultimate glutamine in either a de novo expanded 42 repeat allele or the corresponding non-penetrant 38 repeat allele in a family with fresh mutation to HD.

INTRODUCTION

Huntington’s disease (HD) is a chronic neurodegenerative disorder which is inherited as autosomal dominant and characterized by chorea, dementia and personality disorder (1). The gene responsible for HD contains an expanded and unstable CAG trinucleotide repeat (2).

The high frequency of HD among persons of European descent in comparison with Asian and African populations was considered evidence for a single HD mutation of great antiquity (3). However, since the cloning of the HD gene in 1993, a number of mutation events leading to disease expression have been described (2,4–10). Fathers with repeats larger than those commonly observed in the general population were identified as the transmitting parent in all 12 of the confirmed fresh mutation events [Myers et al. (4); two cases]; Goldberg et al. (5; six cases); De Rooij et al. (11; two cases); Zühlike et al. (7; one case); Davis et al. (9; one case)].

The large alleles observed in relatives of individuals with fresh mutations to HD have been termed ‘intermediate’ alleles (4,5). Intermediate alleles were originally defined as (i) larger than commonly observed in the general population, (ii) not associated with disease expression and (iii) smaller than commonly seen in the HD range (4). We now believe that these alleles expand to the clinical range only in paternal transmission. The distinction between the term ‘premutation’ and intermediate allele rests upon the implication that a premutation, due to its expanded size, is prone to expansion to the clinical range while the ‘intermediate’ allele may or may not have a propensity to expand to the clinical range (4). The term ‘clinical’ allele defines an expanded repeat which is associated with disease expression.

Intermediate HD alleles were first described in the examination of family members of individuals with fresh HD mutations. Goldberg et al. (5) and Myers et al. (4) both reported intermediate alleles among the unaffected parents and siblings in descriptions of de novo HD. Intermediate alleles ranging from 33 to 38 (4), 30 to 38 (5), 32 and 34 (11), 35 (7,9) and 37 (8) repeats have been reported.

Recently, Goldberg et al. (12) have proposed a restriction of the intermediate repeat size to the 30–35 repeat range and contend that individuals with repeats of 36 or larger have alleles in the clinical range (5). However, Rubinsztein et al. (13) reported that 10 of 37 individuals with alleles between 36 and 39 repeats are unaffected at ages greater than 65 years. It is not clear from the Rubinsztein et al. (13) report whether these individuals were from families with de novo expression of HD, were descendants of HD affected persons or were derived from the general population. HD penetrance may be different for individuals sampled from these different sources.

Several large patient surveys have been published and the smallest HD clinical allele has been reported to vary from 36 to 40 repeats while the upper range of normal alleles is reported from 30 to 39 repeats (7,11,14–23). Controversy persists about the significance of alleles between 36 and 39 repeats. For alleles in this size range, there are both individuals reported as HD afflicted as well as unaffected elderly persons with no family history of

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Figure 1. Family #2 represents a large family with de novo HD containing many individuals who survived to old age without neurological impairment among the ancestors of the individual with the fresh mutation (arrow). Age at death is listed below deceased individuals and current age for those living. Individuals from whom blood samples were obtained are indicated by an asterisk. Numbers cited in Table 1 are presented below the asterisk. The pedigree has been modified to conceal the identities of those studied.

HD. Thus the risk of disease for carriers of these alleles and the possibility for reduced penetrance is uncertain. The frequency with which such alleles may expand further into the clinical range is also not established. To address these issues, we have examined the repeat instability associated with paternal transmission of alleles between 37 and 39 repeats for 10 clinically unaffected individuals identified from collateral branches of de novo mutation families.

Finally, Goldberg et al. (12) recently identified a mutation in the glutamine repeat which they propose may be associated with increased repeat instability in a subgroup of HD cases. The penultimate glutamine is coded by a CAA rather than the CAG found for the other glutamines in the repeat. Goldberg et al. (12) observed a mutation of this CAA to CAG which was associated with increased instability in one HD family. We explored whether the CAA has mutated to CAG in a new mutation family and whether this mutation may be associated with disease expression or instability in this family.

RESULTS

Transmission of alleles with 37–39 repeats

We examined the transmission of repeats in the range of 37–39 repeats in two extended families (#2 and #3) previously reported (4).

Family 2. The female proband, a member of a sibship of six, developed HD at the age of 35 and died at age 46. An autopsy confirmed HD. Two of her offspring have developed HD, and repeat studies in the offspring reveal expanded repeats in the clinical range for HD (52 and 48). Both parents were asymptomatic and died at age 79. Death certificates, obituaries and family records were reviewed for all relatives and none had evidence of HD or other neurological impairment (see Fig. 1). The five siblings of the proband are living and aged between 63 and 82. None has symptoms of HD and none of their descendants is affected. Siblings of the proband and the descendants of male siblings were studied.

Table 1. Family 2

<table>
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<th>Repeat sizes</th>
<th>Repeat stability</th>
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<td>2</td>
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<td>18 17</td>
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<td>4</td>
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<td>40–60</td>
<td>19 15</td>
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<tr>
<td>6</td>
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<td>8</td>
<td>70–90</td>
<td>38 15</td>
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Five transmissions: three unchanged, two +1 repeats.

Repeat studies for eight individuals in this family were performed, including the five siblings of the individual with de novo expression and three descendants of a male sibling with 37 repeats (see Table 1).

Family 3. We examined the allele sizes for nine descendants of a man with 38 repeats, who is neurologically unaffected at age 99. An examination of family records, death certificates and obituaries for three generations of ancestors revealed no evidence of HD (see Fig. 2).

When last formally examined at age 97, this individual was living in a nursing facility in a rural community. He was fully oriented to time, place and person and ambulatory with a cane. Primary medical concerns were a moderate hearing loss and osteoarthritis. He continued to be fully independent for activities of daily living and remarkably continued to ride a three wheel bicycle in the community, sometimes making excursions over a mile from the nursing facility. His conversation focused heavily upon recollections of his many years of farming and the trials and tribulations of this occupation. He was lucid and had no difficulty in conversing. He displayed no evidence of involuntary movement and was able to write his name clearly and perform mental arithmetic computations and recall three objects after five minutes.
Figure 2. In Family #3, none of the ancestors of the individual with de novo HD exhibited symptoms of HD. Age at death is listed below deceased individuals and current age for those living. Individuals from whom blood samples were obtained are indicated by an asterisk. Numbers cited in Table 2 are presented below the asterisk. The pedigree has been modified to conceal the identities of those studied.

Table 2. Family 3

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Five paternal transmissions: three unchanged, one −1, one +1 repeat.

The sister of this individual had 42 repeats and was HD afflicted in her early 50s with a diagnosis of new mutation to HD. Autopsy confirmed HD and a daughter, with 44 repeats is affected.

Nine offspring and one grandchild of the 99 year old neurologically normal individual with 38 repeats were studied (see Table 2). Ten individuals with repeats of 37–39 were observed among the 18 persons studied, although none has symptoms of HD, including those surviving into their 70s and 80s.

In addition, a sperm sample of one individual with 39 repeats, from Family 3, was studied for evidence of germ line mosaicism. This study revealed a sperm repeat which is not remarkably different from that seen in the blood specimen (39/17) and thus provides no evidence for instability in gametogenesis (Fig. 3).

Sequencing alleles with 38 and 42 repeats

The glutamine repeats were sequenced in somatic cell hybrids containing a single HD allele. The somatic cell hybrid cell line for the 99 year old unaffected individual, HHW1350, was determined to have 38 CAG repeats and the penultimate glutamine repeat is the expected CAA. The cell lines for the HD affected sister of this individual, HHW1346 and HHW1347, had 42 CAG repeats and the penultimate glutamine is the expected CAA. The analysis confirms that HHW1346 and HHW1347 are two independent hybrids carrying the same chromosome 4 allele.

DISCUSSION

We report 10 transmissions of paternal alleles of 37–39 repeats; five in Family 2 and five in Family 3. All 10 descendants are without symptoms of HD and many are elderly. That individuals with alleles of 37–39 repeats may survive unaffected well beyond common life expectancy supports the notion of reduced penetrance for HD among some persons with repeat sizes which overlap the HD clinical range. While it is possible that some of those with repeats in the range of 36–39 studied here may develop HD later in their lives, the sample includes persons in their 80s and
90s who are neurologically intact. This observation suggests that the designation of the clinical range for genetic testing among the collateral branches of families with apparent de novo expression of HD is uncertain.

In this series, alleles of 37–39 showed instability in four of 10 transmissions, distinguishing them from the extremely low rate of instability observed in normal alleles (14). In the 10 transmissions, one showed contraction of one repeat and three showed expansion of one repeat. Goldberg et al. (12) examined the frequency of repeat instability in 18 paternal transmissions of alleles in a lower range (from 29 to 35 repeats). The rate of instability, eight of the 18 transmissions (seven expansions), is similar to ours.

The frequency of disease expression among paternal transmissions of repeats of 36–39 units in the collateral lineages of fresh mutations cannot be determined with accuracy in this small sample. However, that all 10 survive without evidence of HD suggests that the risk for HD expression is below that expected for descendants of carriers of alleles in the clinical range of 40 or more repeats. Counseling of individuals with repeats between 36 and 39 may need to consider the possibility of different penetrance functions for persons with confirmed ancestry of HD versus those from collateral branches of fresh mutation cases. Additional studies will be necessary to contrast penetrance for these groups.

The mechanisms which predispose to repeat instability and disease expression remain obscure. Recently Goldberg et al. (12) reported a CAA→CAG mutation in the penultimate glutamine of the HD repeat which was associated with increased instability in one family. We examined the sequence of the expanded repeat chromosome for both the de novo expanded allele and the corresponding 38 repeat allele in a family with fresh mutation to HD. Neither of these alleles had the CAA→CAG mutation for the penultimate glutamine. Therefore, this mutation is not related to the instability and disease expression in this family.

Most cases recently reported as potential fresh mutations to HD have not excluded (i) non-paternity, (ii) the possibility that a living unaffected parent has an as yet unexpressed clinical allele, (iii) the early death of one or both parents, or (iv) the possibility that a living unexamined parent may be symptomatic (5,9,10). It is likely that the majority of cases recently reported as potential new mutations have a hidden ancestry of the disease and the frequency of mutation to HD is very low.

We have previously reported (4) that the chromosomal haplotype for both Families 2 and 3 is the major haplotype found in disequilibrium for HD chromosomes. Thus these reduced penetrance alleles of 37–39 repeats are not apparently different from others in this range which are associated with disease expression. The pattern of a preponderance of mutation events associated with a common disease haplotype parallels findings in FMR1, an X-linked CGG trinucleotide repeat disease associated with retardation. In FMR1, one haplotype is over-represented on disease chromosomes (24,25). Thus, for both FMR1 and HD, a majority of mutation events appear to be derived from a limited ancestral source.

We propose that modifying factors influence penetrance for the HD allele. The observation that there is no difference in the sequence of the repeat for those who express disease and those who do not in the 36–39 range, implicates such modifiers, probably including genetic modifiers, which influence disease expression.

Almqvist et al. (26) state that ‘Expansion of a trinucleotide beyond 35 repeats results in a clinical phenotype of HD’. While this statement may be true if the elderly unaffected individuals in this study develop HD later in their lives, the current analysis contradicts this position. The term ‘intermediate repeat’, which we coined in 1993 (4) and which has gained wide usage (5,12) has outlived its usefulness and should be replaced by a range of ‘reduced penetrance’. A reduced penetrance range for alleles between 36 and 39 repeats should be adopted for HD testing protocols although there is no valid ‘cut-off’ to distinguish repeats in the clinical range from those in the normal range. Rather, there is an overlap of repeats sizes, some of which will be associated with disease expression while others will not, depending upon modifying factors. The identification of such modifying factors may represent a fruitful approach to defining therapeutic targets in HD.

**MATERIALS AND METHODS**

**Repeat sequence analysis**

Somatic cell hybrids were created for two individuals in Family 3:

(i) The 99 year old individual with 38 repeats. One somatic cell hybrid line carrying the 38 repeat allele (HHW1350) was studied.

(ii) The individual with de novo expression of HD. Three somatic cell hybrids were studied, two containing the expanded 42 repeat allele (HHW1346, HHW1347) and one containing a normal allele of 17 repeats (HHW1333). HHW1346 and HHW1347 are independent hybrids carrying the same allele.

The cell lines were studied for the polymorphism D4S127 to identify the chromosome 4 carried by each line. The somatic cell hybrids were characterized by PCR of the HD CAG repeat, excluding the adjacent CCG repeat, to determine the size of the allele carried by the cell line (27).

The D4S127 polymorphism has been previously described (28) and has eight alleles.

The HD repeat was studied previously using primers Hu3 (29) and Hu4 (30).

Each DNA specimen was sequenced by the method of Murray (31) by using a [γ-32P]ATP end-labeled Hu3 primer (29). Sequencing was performed using Taq polymerase (Cetus) in the
buffer provided with the addition of dNTPs to a final concentration of 2.5 μM and BSA to a final concentration of 0.01%. The final concentration of ddNTP in the respective reactions was 50 μM ddGTP, 200 μM ddATP, 1 mM ddTTP, and 500 μM ddCTP. Ten PCR cycles were performed at 96°C 30 s, 50°C 90 s and 72°C 120 s. Five microliters of reaction was added to 5 μl of loading dye (formamide), heated to 94°C for 3 min, cooled and loaded on a 6% PAGE run at 70 W constant, until the bromophenol blue reached the bottom of the gel.

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REFERENCES