

Instability of CAG repeats in Huntington's disease: relation to parental transmission and age of onset

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Abstract

Huntington's disease (HD) has recently been found to be caused by expansion of a trinucleotide (CAG) repeat within the putative coding region of a gene with an unknown function. We report here an analysis of HD mutation and the characteristics of its transmission in 36 HD families. CAG repeats on HD chromosomes were unstable when transmitted from parent to offspring. Instability appeared more frequent and stronger upon transmission from a male than from a female, with a clear tendency towards increased size. We have also found a significant inverse correlation ($p=0.0001$) between the age of onset and the CAG repeat length. The observed scatter would, however, not allow an accurate individual prediction of age of onset. Three juvenile onset cases analysed had an HD mutation of paternal origin. In at least two of these cases a large expansion of the HD allele upon paternal transmission may explain the major anticipation observed. Our results suggest that several features of the expansion mutation in HD are similar to those previously observed for mutations of similar size in spinobulbar muscular atrophy and in myotonic dystrophy, and to those observed more recently in spinocerebellar ataxia type 1 and in dentatorubropallidoluysian atrophy, four diseases also caused by expansion of CAG repeats.

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Huntington's disease (HD) is a severe neurodegenerative disorder characterised by progressive motor disturbance, cognitive loss, and psychological changes.¹ It is an autosomal dominantly inherited disease occurring with a frequency of 1 in 10 000 persons in most white populations.² Although the disorder typically has insidious onset around middle age (mean = 38.3 years), a marked variation is observed in age of onset and clinical manifestations.³ Recently, the defective gene in Huntington's disease was identified and was found to contain a trinucleotide repeat (CAG) in the putative coding region.⁴ The repeat is polymorphic in the normal population (11 to 34 CAG repeats) but was found to be expanded beyond 42 units in HD chromosomes.

Thus, HD is the fourth disease found to be caused by expansion of a trinucleotide repeat,

after the fragile X syndrome (CGG repeats in the 5' untranslated region (UTR) of the FMR-1 gene⁵⁻⁷), spinobulbar muscular atrophy (SBMA) (CAG repeats in the coding region of the androgen receptor gene⁸), and myotonic dystrophy (DM) (CTG repeats in the 3' UTR of a gene coding for a putative protein kinase⁹⁻¹¹). Since completion of the present study, three other unstable trinucleotide repeats have been characterised, associated with spinocerebellar ataxia type 1 (SCA1)¹² and with dentatorubropallidoluysian atrophy (DRPLA)¹³ (CAG repeats in both cases), and with the FRAXE fragile site (GCC repeats¹⁴). At least two of these diseases are characterised by increased severity or penetrance of the clinical phenotype in successive generations (called anticipation in DM and 'Sherman paradox' in fragile X syndrome).¹⁵⁻¹⁷ These phenomena result from the tendency for amplification of the repeat when transmitted from parent to child.^{7,11,18,19} However, parental sex biases are observed with respect to the severity of the disease. The congenital form of DM and the transition from premutation to full mutation in the fragile X syndrome both occur only upon maternal transmission.^{7,18,19} In HD it has also been reported that important anticipation occurs in a minority of cases, predominantly when the mutation is transmitted by the father.²⁰ This effect was previously suggested to be caused by genomic imprinting.^{20,21} SCA1 shows a similar pattern of inheritance.²²

In the initial report of the expansion mutation in HD,⁴ it was suggested that age of onset could be inversely correlated with the CAG repeat number. We report here an analysis of HD mutations and the characteristics of their transmission in 36 families, and these features are compared to those observed in the other diseases caused by the expansion of CAG/CTG repeats.

Materials and methods

FAMILIES

Blood samples have been obtained since 1986 through French clinical geneticists, in general for families where an adult at risk had asked for presymptomatic diagnosis or prenatal exclusion diagnosis by linkage analysis. In a very few cases, children were included, only to help characterise parental haplotypes. For some families, blood samples had been conserved for possible later analysis. In all cases, informed consent had been obtained by a clinical geneticist for the use of samples in linkage analysis. Individual results from the present study are

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communicated to clinical geneticists only if specifically requested for adults at risk who ask for presymptomatic diagnosis after proper preparation and information. Our laboratory has no direct contact with families.

PCR DNA ANALYSIS

Genomic DNA was extracted from peripheral blood samples using standard procedures. PCR amplification across the CAG repeat in the HD gene was carried out using the set of primers described previously,⁴ but other conditions were modified. Reaction mixtures contained 10 mmol/l Tris-HCl (pH 8.3), 50 mmol/l KCl, 2 mmol/l MgCl₂, 10% DMSO, 200 µmol/l dATP, 200 µmol/l dCTP, 200 µmol/l dTTP, 50 µmol/l dGTP, 150 µmol/l C⁷-deaza-dGTP and 1 pmol of a 5' end ³²P labelled primer (10 ng), 10 pmol of the opposite unlabelled primer, 200 ng genomic DNA, and 2 units Ampli Taq Polymerase (Perkin-Elmer, Norwalk, CT) in a total volume of 25 µl. Samples were overlaid with mineral oil and subjected to 35 temperature cycles consisting of one minute at 95°C, one minute at 65°C, two minutes at 72°C, followed by a final 10 minute extension at 72°C. Five µl of each reaction mixture were analysed on a 0.4 mm thick 6% w/v acrylamide-8 mol/l urea denaturing gel. PCR products were sized by comparing with the sequence of a fragment containing 45 CAG repeats, cloned from an SBMA patient. For analysis of stability of transmission, samples from the same family were analysed in adjacent lanes of the same gel. Absolute size measurements of HD alleles from different gels may be affected by an uncertainty of one or two repeats. The number of CAG repeats was deduced from the length of the PCR product using the published sequence⁴ and thus reflecting the recently discovered CGG repeat polymorphism.²³

STATISTICAL ANALYSIS

The association between HD allele size and age of onset were examined using linear and polynomial regression and linear regression

with logarithmic transformation of the age of onset. Although all analyses yielded highly significant ($p=0.0001$) association, log transformation of the age of onset appeared to give a better fit based on analysis of the variance and regression coefficients.

Results

DISTRIBUTION OF CAG REPEAT LENGTH ON NORMAL AND HD CHROMOSOMES

To compare the number of CAG repeats at the HD locus in normal and HD chromosomes, PCR DNA analysis was performed on members of 36 unrelated HD families using a protocol modified from the original one⁴ (see Methods). The distribution of 145 independent normal chromosomes and 85 HD chromosomes showed different ranges of CAG repeat length with no overlap (fig 1). Normal chromosomes displayed at least 18 alleles, containing between 13 and 37 repeats with a median of 20 units (mean 20.1, SD 3.5). Sixteen discrete HD sized alleles ranging from 40 to 87 repeats were found and the median was 45 repeats (mean 46.7, SD 6.7). Of the 85 HD chromosomes, 47 were derived from patients with an HD phenotype and 38 were from persons who had no clinical symptoms at the time of blood sampling. This latter group has been included in the HD alleles based on family data (individual results are communicated to genetic counsellors only for those adults at risk who request presymptomatic testing after proper preparation and information (see Methods)). All except four are members of HD families with affected relatives having both a clear HD phenotype and an HD allele. In most cases, previous linkage analysis, notably with VNTRs at D4S43 and D4S125, had shown that they carried an HD mutation. The other four are from families with affected relatives for whom no DNA was available. The persons still free of clinical symptoms are on average younger (mean 26.5, SD 8.2, $n=32$) than the mean age of onset of HD. No sex difference was found for the size distribution of normal or HD alleles (not shown).

The 40 paternally derived HD chromosomes had 40 to 87 CAG repeats with a median of 44 (mean 47.9, SD 8.9) and the 34 maternally derived ones displayed between 40 and 54 CAG repeats with a median of 46 (mean 45.9, SD 3.7). Notably, the three largest alleles (66, 72, 87 units) were derived from the father, suggesting a paternal effect for transmission of such alleles.

EFFECT OF PARENTAL TRANSMISSION ON STABILITY OF CAG REPEATS

Normal CAG alleles were transmitted from parent to offspring without any alteration in size in the 135 cases analysed. However, transmission of HD alleles was associated with a considerable degree of instability (table 1). Among 35 transmissions of an HD allele from parent to offspring, 14 (40%) were found to be unstable. In 30 additional transmissions, the size of the parental HD allele could not be

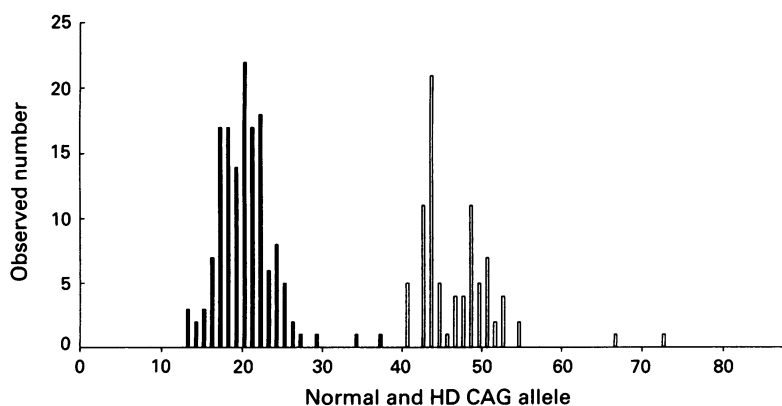


Figure 1 Distribution of allele size on normal and HD chromosomes. One hundred and forty five independent normal chromosomes (filled bars) and 85 HD (open bars) chromosomes were derived from 36 HD families. Number of repeats was calculated by assuming that allele size is dependent only on CAG repeats. Recent observations indicate that variation of up to three trinucleotides may be because of the adjacent CGG repeat.²³

Table 1 Effect of parental transmission on stability of HD allele

	Transmission		
	Total	Stable	Unstable
Observed parent-child pairs	35	21 (0.60)	14 (0.40)
Maternal	21	14 (0.67)	7 (0.33)
Paternal	14	7 (0.50)	7 (0.50)
Deduced parent-child pairs*	30	20 (0.67)	10 (0.33)
Maternal	10	8 (0.80)	2 (0.20)
Paternal	20	12 (0.60)	8 (0.40)
Cumulative transmission	65	41 (0.63)	24 (0.37)

* The degree of stability of CAG alleles was deduced by comparing CAG alleles observed in the offspring of a missing parent when the family contained two or more children. Transmission was considered stable if all children had the same allele length.

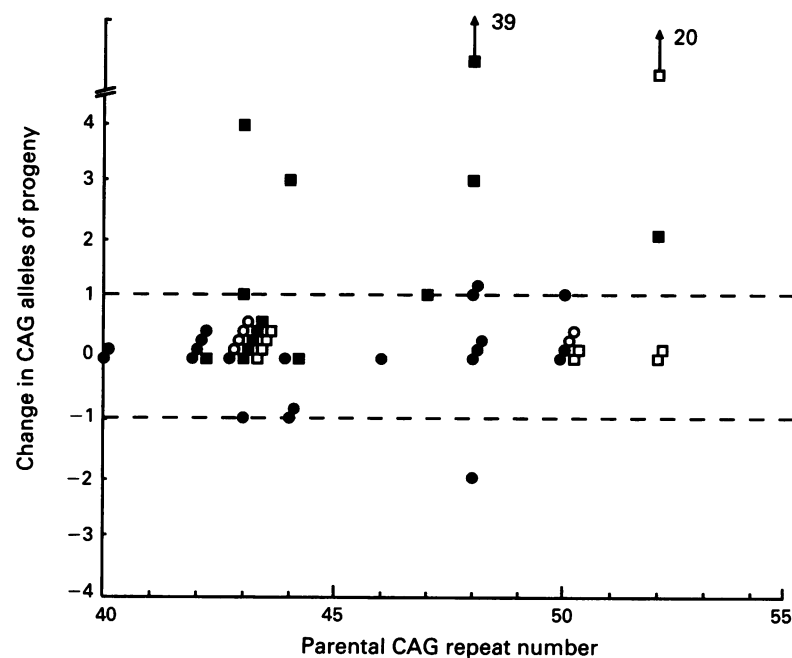


Figure 2 Change in CAG repeat number on HD chromosomes between affected parent and progeny. The alteration of HD allele size in progeny is shown as a function of the sex and repeat number of the affected parent. Twenty seven maternal transmissions (circle) are indicated including six (open circle) transmissions for which the maternal allele was deduced from the repeat number observed in their offspring. Twenty three paternal transmissions (square) are shown including nine transmissions (open square) for which the paternal allele was deduced from that observed in their offspring.

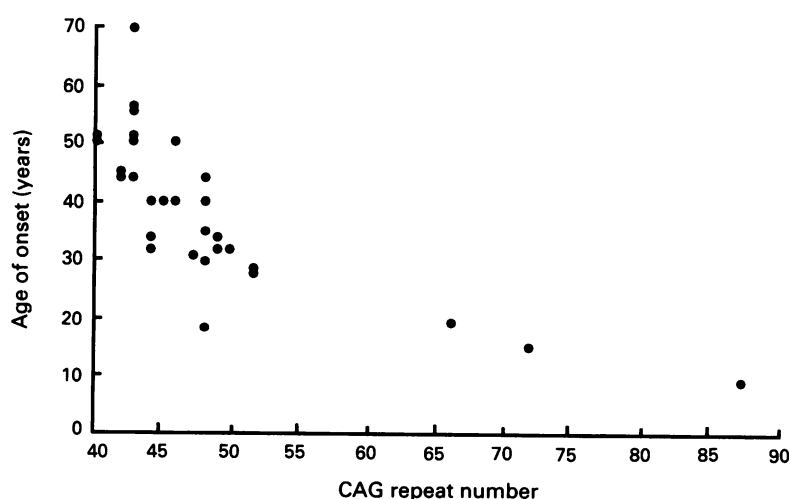


Figure 3 Relationship between CAG repeat number and age of onset. Age of onset of 30 HD patients is shown as a function of the repeat number observed on their HD chromosome.

directly examined but the degree of stability of the transmitted alleles was deduced by comparing the HD allele observed in the offspring when it had been transmitted to two or more children. Even in these additional transmissions, an instability was observed with 10 of 30 (33%) transmissions showing an alteration of allele size. Thus, the overall allele alteration on HD chromosomes was 37% (24/65) of all transmissions analysed. Table 1 also shows the influence of the parental origin of the HD chromosome on the degree of stability of the HD allele. The instability was more frequently seen in paternal transmission (overall, 15 of 34 (45%) transmissions showed instability) than in maternal transmission (overall, 9 of 31 (29%) transmissions were unstable).

The comparison of the CAG repeat number of the HD mutation between affected parent and corresponding progeny is shown in fig 2. Six of 27 maternal transmissions showed alteration (three increases and three decreases) of only one unit between the mother and her child, and one transmission showed a decrease of two units. The average alteration in CAG repeat number in the 27 female meioses was a decrease of 0.1 unit. On 23 paternally derived HD alleles, all the eight alterations of repeat number observed corresponded to an increase in length. Six were of more than one repeat, including two dramatic increases of 20 and 39 units. The average change in CAG repeat number among 23 transmissions from father to child was an increase of 3.2 units. There was no obvious correlation between instability and the size of the parental allele. However, it can be noted that the two largest increases concern parental alleles of 48 and 52 repeats which are above the median value.

An inverse correlation between age of onset and number of repeats was observed (fig 3), which was highly significant ($p=0.0001$) on regression analysis. The best fit was found with linear regression analysis with logarithmic transformation of the age of onset ($r^2=0.79$). Three of the four cases with onset before 20 years had alleles of 66, 72, and 87 repeats respectively and were of paternal origin. In the latter, the child with 87 units was affected at the age of 8 years, while the father (48 units) was affected at the age of 44. The fourth case, with 48 repeats, had a very slowly progressive course of the disease. The inverse correlation was still significant when the three largest alleles (66, 72, and 87 units) were removed from the analysis ($r^2=0.51$, $p=0.0001$).

One family appeared to present a new mutation since the grandparents died unaffected aged 92 and 94 years, while in generation II a single child (dead) manifested the disease at 53 years, and his son was affected at the age of 34 and carried an allele of 49 repeats. In this family, two sisters in generation II are now aged 75 and 76, have no HD symptoms, and carry an allele with 37 repeats, which is at the upper end of the distribution of normal alleles. This allele might represent the original length before the mutation occurred in this family. Analysis with the D4S127 microsatellite and

Table 2 Comparison of allele distribution and stability of CAG repeats at the HD, AR, SCA1, and DRPLA loci

Locus	Normal alleles		Mutated alleles		Transmission instability				
	Mean (SD)	Range	Mean (SD)	Range	% (instabilities/transmissions observed)			Average variation	
					Total	Female	Male	Female	Male
HD gene	20.1 (3.5)	13–37	46.7 (6.7)	40–87	37% (24/65)	29% (9/31)	45% (15/34)	–0.1	3.2
AR gene	20–22*	12–30*	47 (4)†	40–62†	32% (20/62)‡	20% (9/44)‡	64% (7/11)‡	0.1‡	1.2–1.4‡
SCA1 gene	~30	19–36		43–81	Some instability	through paternal	transmission		
DRPLA gene	~15	8–25		54–68	100% (7/7)			–1.0	4.2

* Data from ref 28; † data from ref 29; ‡ cumulative data from ref 29 and 30; SCA1 data from ref 12; DRPLA data from ref 13.

the D4S95 *TaqI* RFLP (these markers are the closest to the CAG repeat⁴), and the more proximal D4S125 VNTR was consistent with, but could not prove, a common origin between the HD mutation and the large normal allele. The more distal marker, D4S93 VNTR, on the other hand, suggested either a different origin or a meiotic recombination (results not shown). The structure of the family (three key persons had died) did not allow us to reach an unequivocal conclusion.

Discussion

Huntington's disease is the fourth reported example of a genetic disorder that results from expansion of an unstable trinucleotide repeat. It is of interest to analyse transmission of the HD mutation and genotype-phenotype correlations, both to understand the genetic features of Huntington's disease better, and also for comparison with the observations in the other expansion diseases, in particular those where the mutation involves a CAG/CTG repeat unit (myotonic dystrophy,^{9–11} spinobulbar muscular atrophy,⁸ spinocerebellar ataxia type 1,¹² and, most recently, hereditary dentatorubral-pallidoluysian atrophy¹³). Such comparisons may clarify those features which are an intrinsic property of the repeat itself, and show whether other factors, such as the flanking sequence or chromosomal environment of the mutation target, or the function of the mutated gene in germ cells or in other cells may influence stability and parental sex biases of transmission.

The distribution of allele size on normal and HD chromosomes in the population we studied is in very good agreement with the results presented in the original report.⁴ In particular, we found no overlap between the sizes of normal and HD alleles, although the difference between the longest normal allele ($n = 37$) and the smallest HD allele found in a patient ($n = 40$) is only three repeats (this interval was from $n = 34$ to $n = 42$ in the previous report). Since completion of this study, three reports have been published on sizing of CAG repeat length at the HD locus in normal persons and HD patients.^{24–26} The largest normal alleles (L) and the smallest disease alleles (S) observed by the three groups were respectively $L = 34$ and $S = 37$,²⁴ $L = 37$ and $S = 38$,²⁶ and $L = S = 34$ and three possible HD alleles with $n = 30–31$.²⁵ Because of the rare apparent overlap between large normal and small HD alleles, one should stress the necessity of very accurate sizing of alleles at that locus for diagnostic purposes. All these analyses included a small

stretch of CCG repeats flanking the CAG motif, which was recently shown to be polymorphic, with major alleles that differ by three repeats.²³ It will be important to determine whether this polymorphism contributes to the apparent overlap between normal and HD alleles.

While normal alleles were found to be completely stable, limited instability of the HD mutation was found in maternal transmission (in most case a change of a single repeat unit) and a more pronounced instability was observed in paternal transmission, with all changes being increases in size (3.2 repeat units on average). Two of 23 paternal transmissions had much larger expansions (20 and 39 units), and the three largest alleles ($n \geq 66$) were all paternally derived. This correlates well with the notion that juvenile onset cases, who make up 5 to 10% of all HD patients, have inherited the disease from their father in most cases, and that 8% of paternally inherited cases show major anticipation of 24.3 ± 9.2 years.^{3,27} These observations are consistent with the inverse correlation between age of onset and mutation size (fig 3).^{4,24–26} The three largest alleles were indeed found in juvenile onset cases. On the other hand, in the $n = 40–52$ range, while there is a statistically significant correlation between allele size and age of onset, the distribution of the latter is wide for identical or very similar allele length (fig 3).^{24–26} Analysis of HD allele size will thus be of limited value for individual prediction of age of onset. The size distribution of normal and disease alleles in the HD gene resembles strikingly that found in the androgen receptor gene in normal and SBMA patients respectively, and that observed most recently at the SCA1 and DRPLA loci (table 2). In the four diseases,^{12,13,29–30} a greater instability in paternal transmission is present (or probable in the case of DRPLA). The lack of alleles larger than 62 in SBMA is in contrast with those observed at the HD locus here and in the initial report⁴ and at the SCA1 locus.¹² However, this lack of large alleles in SBMA may be because of sample size as the cumulative number of SBMA persons reported^{29–31} is about 120. Other explanations could be that such large mutations do not arise at the androgen receptor locus, or that they would lead to a different phenotype from SBMA (for instance, testicular feminisation).¹³

It is more difficult to compare the instability of HD, SBMA, SCA1, and DRPLA mutations to that of similar sized alleles at the DM locus. Disease alleles in DM may reach about 1000 repeats, while alleles in the 50–80 range (called

pre- or protomutations) are associated with at most minimal and late onset manifestations and are found in general in parents of affected children with larger mutations, giving a bias towards apparently higher instability. Single step enlargements of such small mutations up to 600 repeats have been observed in a few cases.^{19,32} In one study where such protomutations were analysed in extended DM families, they could be found transmitted with no or minimal amplification (in 20 of 60 transmissions) and a trend was noted for more frequent and larger instability in male transmission (up to parental protomutations of $n=80$). On the other hand, instability started to be more prominent in the maternal line for parental alleles over 80 repeats.³² It is likely that HD alleles much larger than $n=100$ would be lethal early in life, as the few cases of alleles in the 80–100 range reported up to now lead to very early onset of the disease.

We have observed a new mutation in a family where two members carry an allele at the upper extreme of normal distribution ($n=37$), although the common origin of the mutated allele and the large normal one could not be established. In the original HD report,⁴ two new mutation events were shown to occur from alleles with $n=33$ and 36 repeats. This is consistent with the conclusions, based on linkage disequilibrium studies in fragile X syndrome and myotonic dystrophy, that these diseases are maintained in the population by recurrent mutations occurring on large normal alleles.^{33–35} From our data and those of the initial report,⁴ the frequency of such large alleles at the HD locus can be estimated at about 1% (for $n=33$ to 35). Given the very rare occurrence of new mutations in HD (direct estimated range between 0.5 to 5×10^{-62}) and assuming that the disease frequency is in a state of equilibrium and that patients who have severe juvenile HD (about 5% of all patients) will not reproduce, one can derive a maximum mutation frequency of 5×10^{-4} for HD alleles of $n=33–35$; thus these would not pose a real genetic counselling problem.

Interestingly, a similar frequency of alleles with 30–37 CAG repeats was observed at the DM locus in white populations^{35–37} and the frequencies of HD and DM are comparable in this population. Although the mean and range of normal alleles at the AR locus is very similar to that found at the HD locus, large alleles in the 33–36 range appear extremely rare ($\sim 1/1000^{28,30}$), and this could account for the lower frequency of SBMA (together with significantly decreased fitness owing to reduced fertility of affected males). A similar explanation is likely to account for the rarity of DRPLA¹³ (table 2). Although the frequency of alleles in the 30–36 range is very high at the SCA1 locus, its interruption by the CAT motif is likely to have a stabilising effect,^{38,39} which could account for the low frequency of SCA1.¹²

In conclusion, the present results suggest that the behaviour of the CAG repeat in the HD gene is quite similar to that in the androgen receptor, SCA1, and DRPLA genes, and

perhaps also to that of alleles of comparable size in DM.

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