Linkage disequilibrium and recombination make a telomeric site for the Huntington's disease gene unlikely

Lilias Barron, Ann Curtis, A E Shrimpton, Susan Holloway, Heather May, Russell G Snell, D J H Brock

Abstract

In a Scottish family in which Huntington's disease (HD) was segregating, recombination was observed between the *D4S115/S111* and *D4S43/S95* loci, with the HD gene associated with the more proximal *D4S43/S95* locus. Analysis of linkage disequilibrium in Scottish families showed significant non-random association between the HD gene and alleles at the *D4S95* and *D4S98* loci. This adds to previous evidence that the HD locus is not sited at the telomere of chromosome 4.

Although the Huntington's disease (HD) locus was localised to the short arm of chromosome 4 in 1983,¹ there is still considerable uncertainty about its precise location. Several recombinants have suggested a telomeric site for the mutation,²⁻⁵ which led to efforts to clone the 4p telomere.⁶⁻⁷ Other recombinants point to a more proximal location,^{5,8} and this is supported by linkage disequilibrium data.^{9,10}

In the course of providing presymptomatic testing for HD in Scottish families, we encountered a recombinant adding to the evidence for a non-telomeric location for the HD gene. A review of allele frequencies at marker loci on HD and normal chromosomes, carried out in an ethnically homogeneous Scottish population, confirmed^{9,10} significant

linkage disequilibrium at the more proximal *D4S95* and *D4S98* loci.

Materials and methods

A total of 82 unrelated families, all of Scottish origin and referred to Edinburgh for presymptomatic or prenatal exclusion testing, was used as the source of data for HD chromosomes. For controls the alleles in spouses and those segregating with the non-HD chromosomes were used. Linkage disequilibrium was analysed by χ^2 test with Yates's correction.

DNA polymorphisms examined were 674E-D/*TaqI* and 674E-D/*MboI* (*D4S95*),¹¹ XP500/*MspI* and KP1.65/*BglII* (*D4S43*),¹² 731/*SacI* (*D4S98*), 157.9/*PstI* (*D4S111*),¹² and 252.3/*PstI* (*D4S115*).¹² A further unpublished system, R10/*BamHI*, about 30 kb distal to *D4S98*, was also used.

Results

In the family shown in the figure the HD gene is segregating with the extended haplotype (telomere)-4-C-1-3-D-1-2.3-1.2-(centromere), since this chromosome is found in five affected subjects in three generations (II.4, II.5, III.5, III.6, and IV.5). Only one other affected person was available for typing in this family, IV.3, who had a recombinant HD chromosome, in which the HD gene is associated with the more proximal markers. The haplotypes in this subject's dead affected father (III.3) can be reconstructed from sibs of IV.3 and from other members of the extended family. This suggests that the most likely event was a crossover in a paternal meiosis (III.3) between markers 157.9/*PstI* (*D4S111*) and KP1.65/*BglII* (*D4S43*), with the HD gene proximal to *D4S111*. Polymorphisms at the intervening *D4S98* locus were not informative. Because one of the sibs in generation IV is unaffected at the age of 30 but carries an HD chromosome, individual genders are not shown.

Only one probable haplotype is shown for the dead woman III.4. It has been reconstructed from haplotypes found in her unaffected children, IV.2 and IV.4. However, if the other haplotype in III.4 were

Human Genetics Unit, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU.
L Barron, A Curtis, A E Shrimpton, S Holloway, D J H Brock

Duncan Guthrie Institute of Medical Genetics, Yorkhill, Glasgow.
H May

Institute of Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN.
R G Snell

Correspondence to Professor Brock.

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Family in which HD was segregating. Affected subjects are shaded. Ages of relevant unaffected persons are shown in pedigree symbols. Inferred haplotypes are in brackets. Recombination was observed in IV-3.

(telomere)-1-B-1-3-D-2-2-3-1-2-(centromere), then the A and D alleles at the *D4S43* locus (KP1.65/BglI) in IV-3 could be on opposite chromosomes to those shown proximal to *D4S43*. However, although this possibility cannot be ruled out, it demands the existence of a haplotype in III-4 for which there is no independent evidence.

Linkage disequilibrium data for polymorphisms at *D4S111*, *D4S98*, and *D4S95* are shown in the table. We have excluded results from three other systems in routine use in this laboratory, 252.3/PstI, KP1.65/BglI, and XP500/MspI, since they detect multiple alleles

which are difficult to compare between different Southern blots. In earlier work with seven polymorphisms at the *D4S10* locus, we were unable to find any significant linkage disequilibrium (data not shown).

There is significant non-random association between the HD gene and alleles detected by 674E-D/MboI, 674E-D/TaqI, and 731/SacI (table). We have also included data from 157.9/PstI (*D4S111*) which, although detecting five alleles, is comparatively easy to score, and where the distribution of alleles on HD and normal chromosomes was very similar.

Allele frequencies and percentages on HD and normal (N) chromosomes.

Locus (probe/enzyme)	Allele	HD	N	χ^2 (with Yates's correction)	Relative risk*
		No (%)	No (%)		
<i>D4S95</i> (674E-D/MboI)	1·2	48 (91)	186 (67)	10·913†	4·75
	0·7	5 (9)	92 (33)		
<i>D4S95</i> (674E-D/TaqI)	2·3	24 (51)	96 (34)	4·108‡	1·99
	1·7	23 (49)	183 (66)		
<i>D4S98</i> (731/SacI)	2	15 (27)	29 (11)	8·135‡	2·91
	3	41 (73)	231 (89)		
<i>D4S111</i> (157·9/PstI)	α	1 (3)	1 (0·5)	0·613	
	A	12 (32)	52 (28)		
	B	20 (54)	103 (56)		
	C	4 (11)	28 (15)		
	D	0	1 (0·5)		

*Relative risks for alleles 1·2, 2·3, and 2 being in coupling with the HD gene, respectively.

† $p < 0·01$.

‡ $p < 0·05$.

Discussion

Bucan *et al*¹³ have constructed a physical map of the chromosome region 4p16.3 and ordered DNA markers within three clusters. Those referred to in this paper are: segment I: centromere....*D4S10*; segment II: *D4S95*–*D4S43*–*D4S98*; segment III: *D4S115*–*D4S111*–*D4S90*....telomere. A majority of recombinants between the HD gene and informative markers has placed the HD locus in segment III,^{2–5} with several recombinants indicating that it is telomeric to the distal locus *D4S90*.^{3,4} However, two recombinants indicated a location proximal to *D4S115*,^{5,8} suggesting that the HD locus may be in segment II. In the family analysed in this study a recombinant event was observed in which the HD gene appeared to be segregating with segment II markers.

This finding is supported by linkage disequilibrium data. In two earlier studies,^{10,11} no evidence was found for non-random allele distribution on HD and normal chromosomes using DNA polymorphisms in segments I and III. However, significant linkage disequilibrium was observed at the *D4S95* and *D4S98* loci in segment II. Our results confirm these findings. No disequilibrium was seen at the *D4S10* locus (data not shown) or at the *D4S111* locus in segment III. However, both polymorphisms examined at the *D4S95* locus and one polymorphism examined at the *D4S98* locus showed significantly different allele distributions between HD and normal chromosomes. Where it is possible to make direct comparisons (674E–D/MboI at *D4S95* and 731/SacI at *D4S98*), the same allele is preferentially associated with the HD gene in all three studies. The main difference between this and the two previous studies^{10,11} is that we found significant disequilibrium with the 674E–D/TaqI polymorphism ($p < 0·05$) on HD and normal chromosomes.

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