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

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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, there is still considerable uncertainty about its precise location. Several recombinants have suggested a telomeric site for the mutation, which led to efforts to clone the 4p telomere. Other recombinants point to a more proximal location, and this is supported by linkage disequilibrium data.

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 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 \( \chi^2 \) test with Yates's correction.

DNA polymorphisms examined were 674E-D/TaqI and 674E-D/MboI (D4S95), XP500/MspI and KPI.65/BglII (D4S43), 1231/SacI (D4S98), 157-9/PstI (D4S111), and 252-3/PstI (D4S115). A further unpublished system, R10/BamHI, about 30 kb distal to D4S98, was also used.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)-(4C-1-3-D-1-2-3-1-2-1)-(centromere), since this chromosome is found in five affected subjects in three generations (II.4, II.5, II.15, II.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 KPI.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
Characteristics of the Huntington's disease gene are unlikely to be located in a telomeric site, as linkage disequilibrium and recombination are unlikely. Evidence points to the existence of the genes in question, and haplotypes observed in different pedigrees support this conclusion. Haplotypes observed in pedigrees show a shared pattern, which indicates that the Huntington's disease gene is located on chromosomes that are homologous to those observed in pedigrees. However, the presence of multiple alleles in these pedigrees makes it difficult to compare the linkage disequilibrium data obtained with Southern blotting with that obtained with seven polymorphisms at the D4S10 locus. There is significant non-random association between the HD gene and alleles detected by 674E-D/Mbol, 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.

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

*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

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 studies. The main difference between this and the two previous studies10 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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