Linkage disequilibrium in Huntington's disease: an improved localisation for the gene

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SUMMARY The search for the Huntington's disease gene has recently concentrated on the telomere of the short arm of chromosome 4. The evidence suggesting this position has been based on single crossover events, but there is conflicting evidence regarding the position of the gene relative to the most terminal markers. We have found significant linkage disequilibrium between the markers D4S98 (probe BS731B-C) and D4S95 (probe BS674E-D) and HD, which supports a localisation for the gene proximal to D4S90 and makes a telomeric localisation unlikely. This disequilibrium may also prove to be important in the future in allowing modification of risk estimates based on genetic linkage.

Since the localisation of the gene for Huntington's disease (HD) to the distal short arm of chromosome 4 by linkage to the DNA marker D4S10 (probe G8), a number of strategies have been used in order to obtain more closely linked markers. Most of these new probes have been placed on a physical map of the region. This map consists of three large regions of DNA: region 1, the most proximal, contains the locus D4S10 (G8), region 2, the central region, contains D4S95 and D4S98, and region 3, adjacent to the telomere, includes D4S90 (probe D5) and D4S141 (probe 2R3). The order of the markers has now been independently confirmed by the technique of radiation mapping. The majority of crossover data from family studies has placed the HD gene in the most distal region, close to the telomere. This has led to considerable efforts to clone in the region of the telomere and recently this was reported to have been achieved using a yeast artificial chromosome vector, raising the possibility that the HD gene may be contained within this clone.

Since studies based on single crossovers have yielded conflicting results, and because this approach becomes less informative the closer one approaches the gene, we have looked for linkage disequilibrium between HD and the linked DNA markers. The very low new mutation rate of HD is a necessary condition for the success of this approach. Negative results have already been reported for D4S10 and we report here our findings for several other markers distal to this locus.

Methods

The HD patient samples studied were taken partly from families used for genetic linkage studies and partly from families who had requested genetic prediction. Seventy-six families were used for the study, not all families being informative with all the probes. All were of Caucasian origin, 47% being from Wales and the remaining 53% from various regions of England. The normal chromosomes used to establish non-HD allele frequencies were derived from spouses from HD families and the normal chromosome of affected subjects. The geographical origin of both HD patients and unaffected people was therefore similar.

Results

We have already reported that there is no linkage disequilibrium between D4S10 and HD (table). Similarly, there is no significant disequilibrium between HD and either D4S90 or D4S141, two mar-
The detection of strong linkage disequilibrium between HD and the loci D4S95 and D4S98 is of considerable significance for a number of reasons.

Firstly, it is directly relevant to the localisation of the HD gene and supports a localisation near to D4S95 and D4S98. The lack of disequilibrium at the more distal loci D4S90 and D4S141 also supports this conclusion. The distance from D4S98 to the telomere is at least 2000 kb and taken together all of these results support a proximal location for HD rather than one very close to the telomere as has been suggested. It is not possible to predict the position of the gene more accurately at present, though crossover data suggest that it is probably distal to D4S98. We are currently walking from both probes looking for expressed sequences.

It is difficult to reconcile these new data with the evidence from single crossovers, the majority of which, although in total less than 10, place the HD gene distal to all markers and consequently close to the telomere. Two possible explanations are that double crossovers are relatively common in this region, or alternatively that there is one very large gene or two genes involved and the particular population we have studied has only one mutation at the proximal locus. However, the use of disequilibrium mapping becomes more useful than the single crossover approach as one gets closer to the HD gene because it enables data from all families to be used rather than relying on a decreasing number of informative crossover events.

For linkage disequilibrium to be detected between two markers it is necessary that the markers are not only closely linked but also have very low mutation rates. The latter is known to be the case for HD, but the mutation rates of various RFLPs are less well understood. Our results with D4S95 suggest that the AccI and MboI sites are relatively stable but that the TaqI and PstI sites may be mutable, and hence do not show disequilibrium even though physically very close to the other sites.

A second area of considerable interest will be to see if populations with a widely different frequency of HD (for example, Japan, Finland) show the same association as we have shown here, since this will bear directly on how many loci are involved and how many mutations exist.
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Thirdly, it should be possible in future to use disequilibrium information in genetic prediction, either in conjunction with genetic linkage data, or in isolation when pedigree structure is inadequate. We would stress however that the disequilibrium we have observed with D4S95 and D4S98 is not specific enough for HD chromosomes to allow this to be done at present and that our results may not prove applicable to all populations.

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References


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