

Genetic linkage between Huntington's disease and the DNA polymorphism G8 in South Wales families

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SUMMARY Analysis of the polymorphism shown by the DNA probe G8 in eight South Wales families with Huntington's disease has confirmed close genetic linkage between this marker and the disorder, the most likely genetic distance being two centimorgans (cM). The closeness of the linkage suggests that G8 may have clinical applications in genetic prediction for this condition.

Close genetic linkage has been established between Huntington's disease (HD) and a polymorphic DNA sequence on chromosome 4, G8, as a result of studies in two large HD kindreds, one from the USA and the other from Venezuela.¹ No instances of recombination between marker and disease were initially detected in these families, but recombination has been documented subsequently.² The precise genetic distance and the general applicability of the linkage in HD families need to be firmly established if this linkage is to be applied in clinical practice, and the usefulness of the G8 marker will also depend on the proportion of subjects who are heterozygous (and thus informative), and on the pedigree structure of individual families.

We have studied eight South Wales kindreds with HD whose structure appeared to be capable of giving useful linkage information. They form part of a much larger body of 130 families in South Wales³ which have been extensively documented and are under prospective surveillance.

Methods

Venous blood samples (20 ml) were taken into EDTA, with an additional 10 ml heparinised sample being used for setting up of transformed lymphoblastoid cell lines. Samples studied in Boston were transported immediately by air, DNA being isolated

and cell lines established by methods already described.¹ DNA from samples studied in Cardiff was isolated by the method of Kunkel *et al*⁴ and stored at -20°C before analysis.

A total of 5 µg DNA was digested to completion using the restriction enzyme *Hind*III, and Southern blot analysis was performed as described previously.¹

All affected patients were examined by at least one of us, no family members with equivocal or unknown status being included in the analysis. Unaffected relatives were only included over the age of 40 years, their residual risk of possessing the HD gene being calculated from a life table for age at onset based on our own population.⁵

Genetic linkage analysis of genotype data was carried out by the computer programme LIPED 3.⁶

Results

Fig 1 shows the alleles detected by pKO82 (a subclone of probe G8) with *Hind*III digested human DNA. pKO82 detects two polymorphic *Hind*III sites, the frequency of the rare alleles in 65 normal British subjects being 0.25 and 0.125 for sites 1 and 2, respectively. The four possible combinations of alleles at site 1 and site 2 are called haplotype A, B, C, and D (table 1). Haplotype frequencies observed in the normal British population (n=65) are A 66%, B 9%, C 22%, and D 3%. These are similar frequencies to those observed in 93 unrelated patients affected with HD.

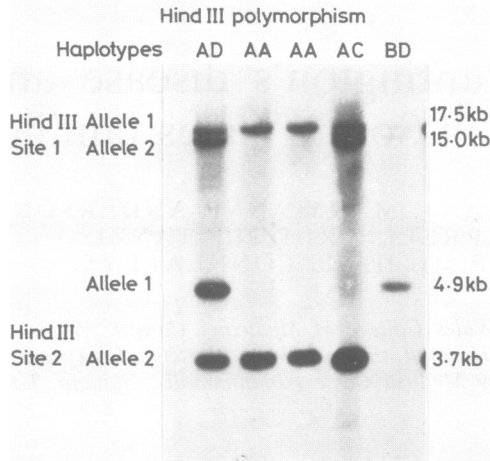


FIG 1 Hybridisation of the probe G8 (subclone pKO82) to HindIII digested human genomic DNA. After digestion of the DNA with the restriction enzyme HindIII, the DNA fragments were separated by electrophoresis on a 0.7% agarose gel. Blotting of the DNA onto nitrocellulose filters was performed according to the method described by Southern.⁷ The haplotypes observed in each subject were assigned as described previously.¹

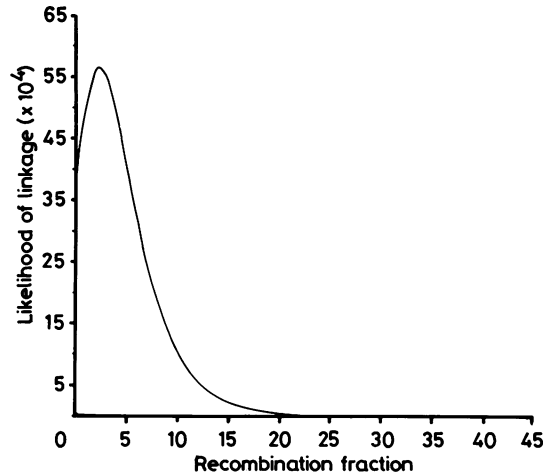


FIG 2 The probability of linkage at various recombination fractions between Huntington's disease and the G8 probe.

TABLE 1 Haplotypes at the G8 locus.

Haplotype	HindIII polymorphism		Haplotype frequency
	Site 1	Site 2	
A	-	+	0.66
B	-	-	0.09
C	+	+	0.22
D	+	-	0.03

+ and - denote presence or absence of the HindIII restriction sites, as designated by Gusella *et al.*¹

TABLE 2 Lod scores for HD and G8.

	Recombination fraction (θ)									
	0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45
Family 1	0.110	0.101	0.087	0.073	0.059	0.047	0.035	0.025	0.016	0.008
2	0.077	0.064	0.049	0.036	0.026	0.017	0.011	0.006	0.003	0.001
3	-0.037	-0.021	-0.006	0.003	0.008	0.010	0.009	0.006	0.003	0.001
4	0.864	0.770	0.647	0.525	0.408	0.300	0.205	0.124	0.062	0.020
5	0.659	0.591	0.508	0.428	0.351	0.279	0.212	0.150	0.094	0.043
6	-0.065	0.326	0.384	0.337	0.256	0.173	0.102	0.052	0.021	0.005
7	0.454	0.396	0.326	0.262	0.204	0.153	0.109	0.072	0.042	0.018
8	3.611	3.340	2.987	2.615	2.223	1.810	1.374	0.924	0.483	0.135
Total	5.673	5.567	4.982	4.279	3.535	2.789	2.057	1.359	0.724	0.231

Table 2 shows the lod scores denoting probability for different recombination fractions (θ); the maximum value of θ is at 2 cM (fig 2) and we have found no evidence of heterogeneity between families.

Fig 3 shows the pedigree of one informative family in which the HD gene is transmitted along with the rare B haplotype. In the other families studied the commonest (A) haplotype was segregating with HD.

Discussion

Our results show that the close linkage between HD and the DNA polymorphism G8, initially reported

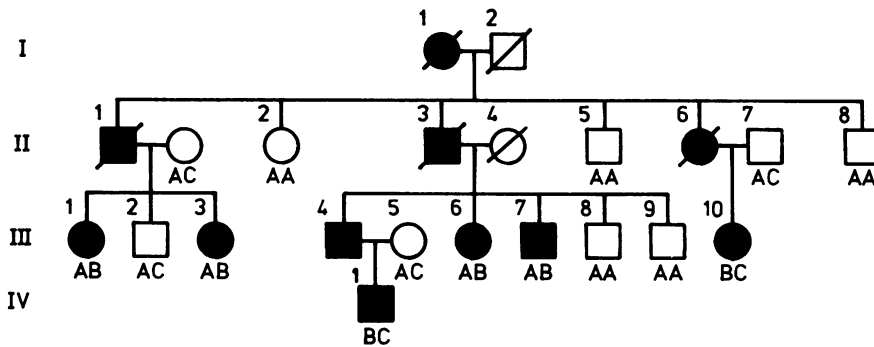


FIG 3 A South Wales kindred with HD, showing transmission of haplotype B with the disorder.

for two large kindreds from the USA and Venezuela, appears to apply also to families in South Wales. Although the relatively small size of these kindreds gives a lower lod score for each, the combined probability is high, with no unequivocal recombination seen between marker and disease. Nor do our families suggest heterogeneity, an important point if this approach is to have general application in prediction.

The population frequency of marker types in the normal UK population appears very similar to that reported by Gusella *et al*¹ for the USA. Combining the *Hind*III with a further three polymorphisms at the G8 locus (using restriction enzymes *Eco*RI, *Nci*I, and *Pst*I),⁸ over 80% of subjects are heterozygous at the G8 locus, and are thus potentially informative for its use in genetic prediction.

Before we can consider using the G8 probe as a predictive marker in families, further work is required to establish the precise genetic distance. The most likely current estimate, based on the original published families, those reported here, and others studied from the USA (Gusella *et al*, in preparation), is approximately 5 cM, suggesting that it will form a predictive test with an accuracy of around 95%. A more accurate prediction will be feasible when markers are available on the opposite side of HD to the G8 locus. Limitations arising from subjects not being heterozygous are likely to be reduced by the discovery of further polymorphisms at the G8 and adjacent loci, so that the major limitation is likely to be the structure of individual families, in particular the absence of living key members such as affected parents or grandparents. The extent of such limitations in the South Wales population is discussed elsewhere.⁹

Study of the G8 probe in chorionic villus biopsies

has shown it to be reliably detectable in such samples,¹⁰ so that this approach is feasible in principle for prenatal prediction in early pregnancy. However, we would not advise clinical application of the linkage until the results of more extensive family studies are available.

Despite these reservations it seems clear that the genetic linkage between HD and G8 is both close and likely to be of general application. It represents an important step towards identification of the HD gene itself and may well prove to be of use in selected families for genetic prediction.

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