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Non-random association between alleles detected at *D4S95* and *D4S98* and the Huntington's disease gene

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SUMMARY Analysis of many families with linked DNA markers has provided support for the Huntington's disease (HD) gene being close to the telomere on the short arm of chromosome 4. However, analysis of recombination events in particular families has provided conflicting results about the precise location of the HD gene relative to these closely linked DNA markers. Here we report an investigation of linkage disequilibrium between six DNA markers and the HD gene in 75 separate families of varied ancestry. We show significant non-random association between alleles detected at *D4S95* and *D4S98* and the mutant gene. These data suggest that it may be possible to construct high and low risk haplotypes, which may be helpful in DNA analysis and genetic counselling for HD, and represent independent evidence that the gene for HD is centromeric to more distally located DNA markers such as *D4S90*. This information may be helpful in defining a strategy to clone the gene for HD based on its location in the human genome.

Directed attempts to clone the gene for Huntington's disease (HD) depend on an accurate knowledge of its location in the human genome. Over the last few years different DNA markers closely linked to the HD gene have been identified.[1–4] Analysis of many HD families with these markers has provided evidence that the gene causing HD lies close to the telomere of the short arm of chromosome 4.[5 6] Currently, attempts are being made to define physically the minimal genomic fragment which contains the HD gene. Towards this goal and in an effort to identify clearly a flanking marker for the gene, recent efforts have been directed towards cloning the telomere of chromosome 4p.[7]

Analysis of families in which recombination between a closely linked DNA marker and the mutant gene has occurred can be extremely helpful in determining the order of the mutant gene relative to these linked DNA markers. We recently reported evidence suggesting the gene for HD was telomeric to the most distal marker *D4S90*[8] based on an analysis of a recombinant in one large family. More recently, contradictory evidence has been presented. MacDonald *et al*[9] have shown by analysis of recombinants in three families that the gene for HD may be flanked by the most distal markers in one family while analysis of two others favours a more telomeric location for the gene.

How can these apparent contradictions be resolved? While analysis of recombination events in selected families is useful in providing evidence in favour of a certain locus order, such studies may also be misinterpreted. One source of error in such analyses may include misdiagnosis of the disease in a crucial family member. In addition, the simplest interpretation of the data is to assume a single recombination event. However, in the absence of a clearly defined distal marker, double recombination events cannot be excluded. This may be particularly relevant as one uses DNA markers close to the telomere where enhanced recombination has already been postulated to occur.[10] Additional independent sources of evidence in favour of a particular locus order relative to the HD gene are needed.

Linkage disequilibrium shown by a non-random association between an allele at one locus with the mutant gene would provide strong evidence that two loci are physically in close proximity. Here we report on the assessment of linkage disequilibrium between six DNA markers [*D4S62* (P8), *D4S10*

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(G8), *D4S95* (674), *D4S98* (731), *D4S96* (678), and *D4S141* (2R3)] and the HD gene. We show that the gene for HD is in significant linkage disequilibrium with *D4S95* and *D4S98*. This provides independent evidence that the gene for HD is proximal to *D4S90* and distal to *D4S10*. The most distally located DNA markers including *D4S90* are therefore likely to flank the gene for HD.

Materials and methods

FAMILIES

A total of 75 unrelated families with a documented history of HD was used in the analysis. All of these families were part of the Canadian Predictive Testing programme for HD. The ancestry of the families was determined during interviews with appropriate family members and is presented in table 1. For controls the alleles in unrelated family members and the alleles segregating with the non-HD chromosome in affected persons were used. In all instances, the ancestry of the family members was assumed to be similar to that of the affected persons. Haplotypes for HD and marker restriction fragment length polymorphisms (RFLPs) were constructed using family studies where phase with HD was unequivocal.

DNA ANALYSIS

A total of 495 persons, 148 affected and 347 unaffected, from these 75 families was included in this analysis. DNA was extracted from leucocytes of each person and digested with restriction enzymes *Hinc*II, *Eco*RI, *Bgl*I, *Hind*III, *Acc*I, *Taq*I, *Mbo*I, *Sst*I, and *Msp*I. Electrophoresis, transfer, and hybridisation conditions were performed as described previously.[11–13] Ten polymorphisms detected by six DNA probes *D4S62*, *D4S10*, *D4S95*, *D4S98*, *D4S96*, and *D4S141* were examined. A *Hinc*II polymorphism was detected by *D4S62*,[2] *Eco*RI, *Bgl*I, and *Hind*III polymorphisms recognised by *D4S10*,[1] *Acc*I, *Taq*I, and *Mbo*I polymorphisms by *D4S95*,[3] a *Sst*I polymorphism by *D4S98*,[14] and an *Msp*I polymorphism by *D4S96*.[14] Two polymorphisms are detected by *Acc*I.[3] The *Acc*I polymorphism assessed in this analysis was the result of a single site variation and resulted in fragments of 6·8 and/or 1·5 kb. *D4S141* was isolated from the same cosmid as recognised by *D4S90*.[15] A frequent *Hind*III polymorphism was used in this analysis.

The order of these DNA markers has been previously established as *D4S62-D4S10-D4S95-D4S98-D4S96-D4S90*-telomere.[5 6 8 9] A diagramatic map with approximate genetic distances between these DNA markers is shown in fig 1. By convention, for single site variation polymorphisms, the larger band is termed A and the smaller band B (fig 2).

TABLE 1 *Ancestries of affected persons in 75 separate families with HD.*

	No
United Kingdom	
England	21
Ireland	6
Scotland	10
Wales	0
British (exact ancestry unknown)	4
Total	41
Non-United Kingdom	
Egypt	1
French Canadian	4
Germany	5
Holland	2
Pennsylvania Dutch	1
Hungary	1
Indian (Asian)	2
Metis (North American Indian)	1
Italy	1
Mennonite Dutch	1
Mennonite Russian	2
Norway	2
Romania	1
Russia	2
Sweden	1
Grand total	27
Ancestry unknown	7
Grand total	75

STATISTICAL ANALYSIS

Non-random association was evaluated by χ^2 analysis of allelic counts for locus pairs. Significant association for biallelic pairs was determined by Fisher's exact probability test (one tailed). The linkage disequilibrium metric, r, was used to measure the magnitude of non-random association where $r=(g-p_1p_2)(p_1q_1p_2q_2)^{1/2}$ and pj and qj are the frequencies of the alternative alleles at site j, and g is the observed frequency of the p_1p_2 haplotype.

Results

The non-HD chromosome allelic frequencies for the 10 polymorphisms assessed are in general agreement with past studies[1–4] and are shown in table 2. The frequencies of the alleles for the HD chromosomes are similar to their unrelated family member

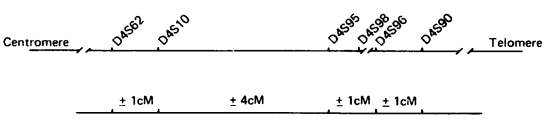

FIG 1 *A diagramatic map showing approximate genetic distances between DNA markers used in this study. (Based on data presented in references 5, 6, 8, and 9).*

TABLE 2 *Allele frequencies and percentages for RFLPs on HD and non-HD chromosomes.*

		HD chromosomes		*Non-HD chromosomes*		*r*	*p value*
		No	%	*No*	%		
D4S62 (P8) (*Hinc*II)	A	25	62·5	115	71·9	0·07	NS
	B	9	22·5	30	18·8		
	C	6	15·0	14	8·7		
	D	0		1	0·6		
	Total	40	100·0	160	100·0		
D4S10 (G8) (*Eco*RI)	A	17	44·7	73	49·0	−0·03	NS
	B	21	55·3	76	51·0		
	Total	38	100·0	149	100·0		
D4S10 (G8) (*Bgl*I)	A	18	60·0	93	65·5	−0·04	NS
	B	12	40·0	49	34·5		
	Total	30	100·0	142	100·0		
D4S10 (G8) (*Hind*III)	A	24	85·7	103	72·0	0·08	NS
	B	1	3·6	13	9·1		
	C	3	10·7	24	16·8		
	D	0		3	2·1		
	Total	28	100·0	143	100·0		
D4S95 (674) (*Taq*I)	A	15	27·3	76	31·0	−0·03	0·35
	B	40	72·7	169	69·0		
	Total	55	100·0	245	100·0		
D4S95 (674) (*Acc*I)	A	54	80·6	175	63·6	0·14	0·005
	B	13	19·4	100	36·4		
	Total	67	100·0	275	100·0		
D4S95 (674) (*Mbo*I)	A	39	76·5	107	56·9	0·16	0·007
	B	12	23·5	81	43·1		
	Total	51	100·0	188	100·0		
D4S98 (731) (*Sst*I)	A	6	25·0	7	7·6	0·22	0·026
	B	18	75·0	85	92·4		
	Total	24	100·0	92	100·0		
D4S96 (678) (*Msp*I)	A	27	56·3	128	59·8	−0·03	NS
	B	21	43·7	86	40·2		
	Total	48	100·0	214	100·0		
D4S141 (2R3) (*Hind*III)	A	5	22·7	28	39·4	−0·15	NS
	B	17	77·3	43	60·6		
	Total	22	100·0	71	100·0		

NS=not significant at p≦0·05.

TABLE 3 *AccI and MboI alleles of HD and non-HD chromosomes by ancestry.*

*Acc*I: United Kingdom

	English		Irish		Scottish		British		Total			
Alleles	*HD*	*non-HD*	*HD*	*non-HD*	*HD*	*non-HD*	*HD*	*non-HD*	*HD*	*non-HD*	*r*	*p*
A	14	43	5	14	7	30	4	11	30	98	0·16	0·02
B	4	26	0	10	2	11	0	8	6	55		

*Acc*I: Non-United Kingdom

	French Canadian		German		Other		Total			
Alleles	*HD*	*non-HD*	*HD*	*non-HD*	*HD*	*non-HD*	*HD*	*non-HD*	*r*	*p*
A	8	14	3	7	11	38	18	59	0·09	0·23
B	0	2	1	8	4	23	6	33		

*Mbo*I: United Kingdom

	English		Irish		Scottish		British		Total			
Alleles	*HD*	*non-HD*	*HD*	*non-HD*	*HD*	*non-HD*	*HD*	*non-HD*	*HD*	*non-HD*	*r*	*p*
A	9	24	4	7	5	21	4	9	22	62	0·19	0·02
B	4	21	0	8	2	2	0	8	6	50		

*Mbo*I: Non-United Kingdom

	French Canadian		German		Other		Total			
Alleles	*HD*	*non-HD*	*HD*	*non-HD*	*HD*	*non-HD*	*HD*	*non-HD*	*r*	*p*
A	4	11	2	0	5	17	11	28	0·10	0·30
B	0	1	1	6	4	14	5	21		

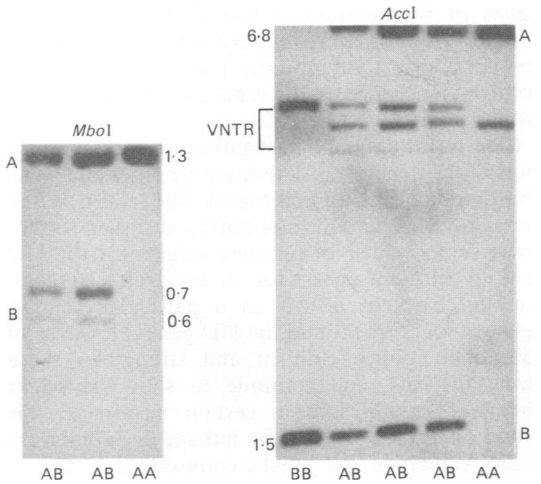

FIG 2 *The polymorphisms of AccI and MboI detected by D4S95. A refers to the larger fragment (6·8, 1·3 kb) while B pertains to the smaller fragments (0·7, 0·6 kb).*

TABLE 4 *AccI and MboI haplotype frequencies in persons affected with HD and their relatives.*

	HD chromosome		*Non-HD chromosome*		*r*	*p*
	No	%	*No*	%		
A (*AccI*) A (*MboI*)	39	76	91	55	0·15	0·01
AB	0		12	7		
BA	0		1	1		
BB	12	24	62	37		
Total	51	100	166	100		

controls for the polymorphisms detected by *D4S62*, *D4S10*, *D4S96*, and *D4S141*. However, two of the three RFLPs (*Acc*I and *Mbo*I) (fig 2) detected by *D4S95* show statistically significant differences (p=0·005 and p=0·007, respectively) in allelic frequencies between HD chromosomes and controls. The third RFLP (*Taq*I) detected by *D4S95* shows no allelic frequency difference between HD and non-HD chromosomes (p=0·35). The polymorphism detected by *D4S98* is also in linkage disequilibrium with HD (p=0·03).

The families in this study represent many different ancestries (table 1). A significant difference for *Acc*I (p=0·02) and *Mbo*I alleles (p=0·02) on HD and non-HD chromosomes was seen in families emanating from the United Kingdom. However, for the smaller number of families outside the UK the trend in allele frequencies was similar but was not statistically different (table 3).

As noted previously,[3] the single site variation polymorphism detected by *Acc*I is in strong linkage disequilibrium with *Mbo*I. For these two RFLPs the A alleles cosegregate as do the B alleles. Thirty-nine of 51 HD chromosomes have an AA haplotype and 12 have a BB haplotype. No AB or BA haplotypes were detected in this group (table 4). It was possible to determine the phase of the *Acc*I and *Mbo*I polymorphism segregating with HD in 67 and 51 of 75 families assessed respectively (table 4). There were significant differences in the haplotype frequencies between HD and non-HD chromosomes (p=0·01) (table 4) and the families from the UK when analysed as a separate group (p=0·02) (table 5).

TABLE 5 *AccI and MboI haplotypes of HD and non-HD chromosomes by ancestry.*

United Kingdom

	English		Irish		Scottish		British		Total					
Haplotypes	*HD*	*Non-HD*	*HD*	*Non-HD*	*HD*	*Non-HD*	*HD*	*Non-HD*	*HD*	%	*Non-HD*	%	*r*	*p*
AA	9	20	4	7	5	20	4	7	22	79	54	54	0·18	0·02
AB	0	4	0	2	0	2	0	1	0	0	9	9		
BA	0	0	0	0	0	0	0	1	0	0	1	1		
BB	4	16	0	6	2	9	0	5	6	21	36	36		
Total	13	40	4	15	7	31	4	14	28	100	100	100		

Non-United Kingdom

	French Canadian		German		Other		Total					
Haplotypes	*HD*	*Non-HD*	*HD*	*Non-HD*	*HD*	*Non-HD*	*HD*	%	*Non-HD*	%	*r*	*p*
AA	4	11	2	0	5	16	11	69	27	56	0·09	0·34
AB	0	0	0	0	0	2	0	0	2	4		
BA	0	0	0	0	0	0	0	0	0	0		
BB	0	1	1	6	4	12	5	31	19	40		
Total	4	12	3	6	9	30	16	100	48	100		

Ancestry unknown

Haplotypes	*HD*	%	*Non-HD*	%	*r*	*p*
AA	6	86	10	56	0·30	0·21
AB	0	0	1	5		
BA	0	0	0	0		
BB	1	14	7	39		
Total	7	100	18	100		

However, there is a similar trend but no significant difference in the haplotype distributions of HD and non-HD chromosomes for the families from outside the United Kingdom (p=0·34) and for families with unknown ancestry (p=0·21) (table 5).

Discussion

The major finding of this study is the non-random association between alleles for *Acc*I and *Mbo*I detected by *D4S95*, *Sst*I alleles for *D4S98*, and the mutant gene for HD.

Linkage disequilibrium was detected with *Mbo*I and *Acc*I and not with *Taq*I with *D4S95*. Possible explanations for this include the presence of a recombinational hot spot between the sites for *Taq*I on the one hand and *Mbo*I and *Acc*I polymorphisms on the other. Alternatively it has already been shown that the recognition site for *Taq*I itself may act as a mutational hot spot[16] with the *Taq*I polymorphism arising independently on several occasions. Finally it is possible that there are multiple origins of the HD gene on identical *Acc*I and *Mbo*I haplotypes but different *Taq*I backgrounds. In all these instances linkage disequilibrium would not be apparent with *Taq*I.

The 75 families in this study are of many different ancestries with the majority being of British descent (41/75). The other 34 families include at least 15 different ancestries. The allele frequencies of *Acc*I and *Mbo*I show significant differences between HD and non-HD chromosomes for the whole group and for families from the United Kingdom. A similar trend, but which with smaller numbers does not show statistical significance, was seen with the non-UK families. It is particularly noteworthy that the haplotype distributions do not significantly differ between UK and non-UK families.

HD is a disease with an extremely low mutation rate. The frequency of the disease in persons of Caucasian descent varies between 5 and 7/100 000, while there is a particularly low prevalence in persons of Black and Japanese descent.[17] These findings, together with accumulating genealogical evidence which traced families in Australia,[18] South Africa,[19] and North America[20] to Europe, implicates north western Europe as the major source of the gene and suggests that there may have been only a few separate mutations underlying the frequency of HD in the world.[17] The finding of similar haplotype distributions in both British families and other families of many different ancestries is suggestive evidence against many different mutations underlying HD. On the contrary this would suggest that there may be only a few HD mutations which have spread around the world during particular waves of immigration. These findings, however, must be viewed as preliminary and do not exclude the possibility that multiple mutations may have occurred by chance on the same chromosomal background.

Clearly, additional investigations in certain ethnic groups are needed to determine whether the same non-random association is found. The failure to find the same non-random association in other studies may occur because of separate origins for the HD gene in different ancestries, or may reflect recombination early in history in a particular country between markers around the HD gene. However, if additional studies confirm and strengthen these initial findings, and continue to show particular linkage disequilibrium in certain ancestries, this would open the way to using linkage disequilibrium in DNA analysis and genetic counselling for HD in certain situations. It may be possible to calculate high and low risk haplotypes.

Over 80% of persons requesting predictive testing for HD are able to receive a significant change in risk.[21] Our major limitation in performing DNA analysis for such persons is the unavailability of DNA from crucial family members, which excludes up to 20% of persons from receiving a modification of risk. The ability to detect heterozygosity in an affected person is not now a problem if multiple highly polymorphic markers are used.[21] Linkage disequilibrium data, if confirmed, may significantly reduce the need for DNA from numerous relatives and may allow predictive testing to be performed even in the absence of DNA from an affected person in certain instances. Such linkage disequilibrium data are now frequently used for risk calculation in some cystic fibrosis families where it has improved estimates of risk.[22]

These HD haplotype studies are similar to investigations in cystic fibrosis in that for both diseases the mutant gene is not cloned. In both instances linkage disequilibrium data can be used to provide clues as to the location of the mutant gene.[23]

In this study, non-random association was not seen with four other polymorphic DNA markers including *D4S62*, *D4S10*, *D4S96*, and *D4S141*. In particular no evidence for linkage disequilibrium was seen using distal markers *D4S96* and *D4S141*. The absence of disequilibrium does not necessarily imply that it does not exist but rather that it was not detected. The sample sizes in this study are small compared with those that are absolutely necessary for a definite statement that disequilibrium does not exist.

Nevertheless some conclusions based on the data presented can be made. In particular, these findings suggest that the gene for HD may be more proxi-

mally located than we,[8] and others,[6] have previously reported and may be centromeric to some of the more distally located DNA markers such as *D4S90* or *D4S141*. A reasonable explanation for the apparent contradictory results concerning the location of the HD gene is that double recombination has occurred. Currently, in the absence of a polymorphic sequence adjacent to the telomere, this hypothesis remains untested. Nevertheless these findings suggest that directed cloning strategies to identify the gene for HD should not be concentrated only on the most distal region close to the telomere on chromosome 4.

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