

Significant linkage disequilibrium between the Huntington's disease locus and markers at loci D4S10, D4S95, and D4S111 in Northern Ireland

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Abstract

An analysis of the Northern Ireland Huntington's disease (HD) population of 75 families showed significant linkage disequilibrium between the HD gene and DNA markers at D4S95, D4S10, and D4S111. As the linkage disequilibrium at loci D4S10 and D4S111 is different from previous studies in the UK, but similar at locus D4S95, this suggests either that the HD mutation(s) in the Northern Ireland and British populations is not of common origin or that the haplotype of the common HD mutation has changed over time subsequent to divergence from a common origin.

(*J Med Genet* 1993;30:1018-19)

HD is a progressive neurodegenerative autosomal dominant disorder with movement, cognitive, and psychiatric dysfunction. Recent research has shown that the condition is caused by expansion of a trinucleotide repeat (CAG)_n in the IT15 gene.¹ As the clinical onset of HD is later in life, there has been difficulty in genetically defining clinically unaffected subjects in families. This had made haplotyping and conventional linkage studies difficult and at times misleading. Alternative methods were used to help localise the HD gene, including linkage disequilibrium. Significant linkage disequilibrium has been shown between the HD

gene, the locus D4S95, and other markers.²⁻¹⁰ In Northern Ireland, 75 unrelated HD families were analysed with several markers in the HD gene region (4p16.3-pter).

Materials and methods

DNA was extracted using either an automated DNA extractor (Applied Biosystems, USA) or manually.¹¹ Southern blot analysis was carried out on the genomic DNA for the D4S95 locus.¹² Polymerase chain reaction (PCR) was used to analyse five multiallelic markers: IDUA at locus D4S111,¹³ YNZ32 at locus D4S125 (the fragments obtained were not further digested),¹⁴ 157.9 at locus D4S111,¹⁵ Kp1.65 at locus D4S43,¹⁶ and G8 at locus D4S10.¹⁷ The PCR cycles used are shown in table 1. Multiallelic PCR products are shown in fig 1. Using the original primer sequences,¹⁸ the PCR product from probe G8 was digested with *Hind*III to show the *Hind*III site 1 polymorphism, giving band sizes of 93 bp (undigested) and 59 bp (digested).

For the allele frequency study, samples from clinically affected HD patients were used. Phase was determined by typing affected HD patients and their parents. DNA markers for normal chromosomes were determined by typing spouses of HD families where possible. All patients sampled had Northern Ireland ancestry. Linkage disequilibrium was calculated using a χ^2 statistic with Yates's correction.

Results

Table 2 shows the linkage disequilibrium data for HD and normal chromosomes. Highly significant results were obtained with the markers D4S10/G8/*Hind*III ($p=0.0006$), D4S95/p674/*Mbo*I ($p=0.0063$), and D4S111/IDUA/*Kpn*I ($p=0.0047$). Other markers, including D4S95/p674/*Taq*I, did not show significance. Fig 2 shows the location of probes used on chromosome 4.¹⁹

Discussion

In this study linkage disequilibrium has been detected at loci D4S10, D4S95, and D4S111 in the HD patient group. Previous studies from the UK²⁻⁴ and in Canadian families with British ancestry,⁵ have shown significant linkage disequilibrium at D4S95 but not at D4S10. The region around D4S95 shows consistent linkage disequilibrium in all popula-

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Received 22 March 1993.
Revised version accepted 5 August 1993.

Table 1 PCR cycle settings.

G8	5'@ 94°C 31 × (1'@ 94, 1'@ 56, 0.5'@ 72)
Kp1.65	4'@ 95°C 1 × (1'@ 95, 1'@ 58, 0.5'@ 72) 30 × (1'@ 95, 2'@ 55, 1'@ 72)
IDUA	5'@ 95°C 30 × (0.5'@ 95, 0.7'@ 66, 0.7'@ 72)
157.9	7'@ 96°C 30 × (0.5'@ 95, 0.7'@ 65, 1.5'@ 72)
YNZ32	5'@ 95°C 10 × (1'@ 94, 2'@ 65, 3'@ 72); 15 × (1'@ 94, 2'@ 60, 3'@ 72); 5 × (1'@ 94, 2'@ 57, 3'@ 72)

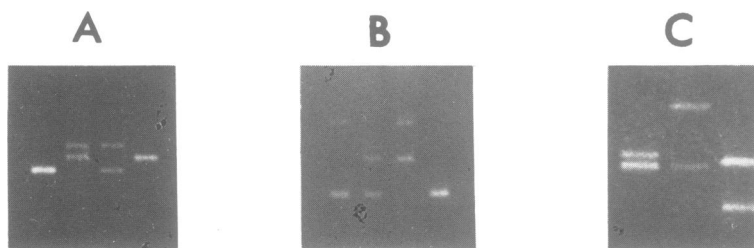


Figure 1 Multiallelic PCR markers used in this study visualised by ethidium bromide staining in agarose gels. Gel A (1.2% BRL agarose) shows 157.9 (D4S111) with alleles at 630, 715, and 800 bp. Gel B (1% Nusieve, 1% BRL agarose) shows Kp1.65 (D4S43) with alleles at 184, 268, and 338 bp. Gel C (1.2% agarose) shows YNZ32 (D4S125) with the largest allele ~ 2 kb.

Table 2 Distribution of alleles for the markers D4S10, D4S125, D4S95, D4S43, and D4S111 in HD and normal chromosomes.

Locus	Probe	Allele	HD	Normal	χ^2	p				
D4S10	G8 (HindIII)	1	39	34	11.846, df 1	0.0006				
		2	0	12						
D4S125	YNZ32	A	0	1	9.710, df 8	0.2860 (NS)				
		B	0	2						
		C	3	7						
		D	13	8						
		E	19	23						
		F	5	12						
		G	6	7						
		H	1	5						
		I	1	0						
D4S95	674ED (TaqI)	1	10	37	0.342, df 1	0.5585 (NS)				
		2	16	77						
	(MboI)	1	23	85						
		2	2	47						
D4S43	Kp1.65	1-3	1	3	7.993, df 7	0.3332 (NS)				
		4	4	7						
		5	8	3						
		6	2	2						
		7-8	1	1						
		9	32	17						
		10	4	2						
		11	41	17						
		D4S111	IDUA (KpnI)	1			4	19	8.010, df 1	0.0047
				2			31	29		
			157.9	1			4	14		
2	19			41						
3	1			4						
	4	0	1	1.217, df 3	0.7490 (NS)					

NS = not significant

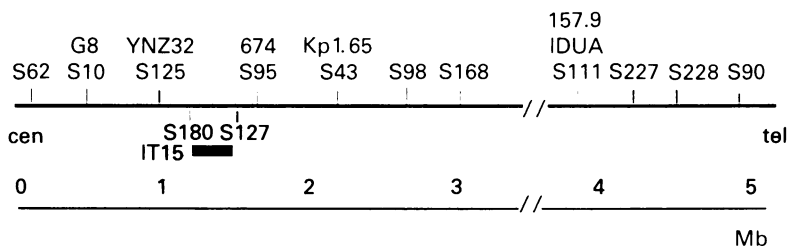


Figure 2 DNA markers around the HD gene locus on chromosome 4. The markers used in this study are shown above their appropriate locus number. The HD gene is marked with a bold line.

tions, suggesting a region of stability. All populations showing significant linkage disequilibrium with D4S95 (*MboI*) and D4S10 (*HindIII*) show an association with allele I. The Italian population⁷ and the Dutch population⁹ also show linkage disequilibrium at the D4S10 locus, the latter over the region from D4S10 to D4S111, similar to our own study. Studies in mixed populations have shown linkage disequilibrium at D4S95 and also in the region D4S133/D4S228, 3 Mb telomeric to D4S95.²⁰ The IT15 (huntingtin) gene has been isolated from the region between the markers D4S180 and D4S127 (fig 2), adjacent to the locus D4S95, which has shown consistent linkage disequilibrium in all populations. However, regional variations in linkage disequilibrium identified at loci as distant as

D4S10 and D4S228, together with evidence from haplotype analysis,⁸ imply that there are multiple origins of this triplet repeat expansion mutation and thus that HD has not originated from a single ancestral gene. More extensive haplotyping of HD chromosomes will now be possible because of the ability to determine repeat number in affected and at risk family members. This should enable more accurate regional HD haplotypes to be identified and help in localising founder origins of HD mutations in Europe and patterns of genetic drift.

PJM is supported by a Royal College of Physicians of Ireland Glaxo Fellowship in Molecular Biology. This work was supported in part by a grant from the Mental Health Foundation.

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