A LINKAGE STUDY WITH DNA MARKERS (D4S95, D4S115, AND D4S111) IN JAPANESE HUNTINGTON DISEASE FAMILIES

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Summary Attempts to isolate the Huntington disease (HD) gene based on its position have been frustrated by apparently contradictory recombination events in HD pedigrees that have predicted two non-overlapping candidate regions: 100 kb at the telomere of the short arm of chromosome 4, and a 2.2 Mb region located internally at 4p16.3. The proximal location is also supported by the detection of a linkage disequilibrium between HD and some restriction fragment length polymorphisms (RFLPs) at the D4S95, D4S98, and D4S127 loci. In the present study, a proximal marker D4S95 showed tight linkage to the disease locus in Japanese pedigrees ($Z_{\max}=3.31$, $\theta_{\max}=0.00$), while distal markers D4S115 and D4S111 did not. Particularly, a two point linkage analysis between D4S111 and HD yielded a lod score -2.01 for $\theta=0.015$. This result leads to the exclusion, as a possible region of localization of the HD gene, of more than 3 cM of the genome around D4S111 locus. At the same time our results favor aforementioned proximal location as a candidate location for the HD gene.

Key Words Huntington disease, linkage analysis, D4S95, D4S115, D4S111

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INTRODUCTION

Huntington disease (HD) is a progressive neurodegenerative disorder that is inherited as an autosomal dominant and highly penetrant trait. The three cardinal symptoms of this disorder are (1) choreic movements, (2) mental defect, and (3) change in personality. The disease usually begins between the age of 30 and 45 years and progress unremittingly for 15–20 years. The neuropathologic hallmark of the disorder is a marked atrophy of the basal ganglia, particularly of the caudate nucleus. However, little is known about the biochemical basis of the neuronal cell death in the disorder.

Genetic linkage analysis with DNA marker D4S10 (G8) mapped HD to the short arm of chromosome 4 (Gusella *et al.*, 1983, 1985). Multipoint linkage analysis established that the HD gene is 4 cM distal to D4S10 and is located at 4p16.3, the terminal cytogenetic subband of chromosome 4 (Gilliam *et al.*, 1987; MacDonald *et al.*, 1987). All subsequent families tested showed linkage to this region (Coneally *et al.*, 1989), suggesting that a single locus is mutated in all individuals affected with the disease. D4S111 and D4S115 were derived from a *NotI* linking library generated from a somatic cell hybrid, HHW693 (Pohl *et al.*, 1988). Both loci show multi-allele RFLPs of variable number of tandem repeat (VNTR) type (Nakamura *et al.*, 1987). Each of these loci was tightly linked to the HD gene in a large Ven-

Fig. 1. Placement of DNA marker loci on the physical map of 4p16.3 (modified from MacDonald ME *et al.*, 1989A). D4S95 is located in the proximal candidate location, while both D4S115 and D4S111 are distal to it.

ezuelan kindred and several smaller pedigrees from the U.S. and Europe, and was tightly linked to the other locus, with no recombinants in 179 informative meioses in reference pedigrees (MacDonald *et al.*, 1989a). Complementary genetic (MacDonald *et al.*, 1989b; Youngman *et al.*, 1989; Allito *et al.*, 1991) and physical (Bucan *et al.*, 1990) (Fig. 1) maps of 4p16.3 were generated. In these maps, D4S115 and D4S111 are 250 kb apart and are located distal to D4S98/S114/S113 (Pohl *et al.*, 1988), while D4S95 is proximal to it (Fig. 1). Recombination events with HD have not decisively positioned the HD gene but suggest two non-overlapping locations (MacDonald *et al.*, 1989b). Three events indicated a location close to the telomere, distal to all known RFLP markers (Fig. 1, the distal candidate location) (MacDonald *et al.*, 1989b; Robbins *et al.*, 1989). In contrast, a more proximal location was defined at its distal boundary by a recombination between D4S43 and D4S98 (Fig. 1, the internal candidate location) (Snell *et al.*, 1992). The proximal location was also supported by the detection of linkage disequilibrium between HD and some RFLPs at the D4S95, D4S98, and D4S127 loci (Snell *et al.*, 1989; Theilmann *et al.*, 1989; Adam *et al.*, 1991; MacDonald *et al.*, 1991, 1992). Recently, a new gene, IT15, isolated from the proximal candidate location contains a polymorphic trinucleotide repeat that is expanded on HD chromosomes (The Huntington's Disease Collaborative Research Group, 1993).

In this study, we analyzed 18 Japanese kindreds using these two distal markers (D4S115 and D4S111) and D4S95.

MATERIALS AND METHODS

Subjects. Families of documented history of HD were ascertained (Karazawa *et al.*, 1990). A total of 18 Japanese kindreds were examined for linkage between the HD gene and the DNA markers D4S95, D4S115, and D4S111.

DNA probes. The following chromosome loci were used in the linkage study: D4S95, probe BS674 which detects *AccI*, *MboI*, and *TaqI* polymorphisms (Wasmuth *et al.*, 1988; Smith *et al.*, 1988); D4S111, probe p157.9 which detects *PstI* polymorphism (MacDonald *et al.*, 1989a; Whaley *et al.*, 1988); D4S115, probe p252.3 which detects a *PstI* polymorphism (Bucan *et al.*, 1990; Poustka *et al.*, 1987).

DNA and hybridization. Epstein-Barr virus-transformed lymphoblastoid cell lines and cultured peripheral blood lymphocytes were used as a source DNA from members of the 18 Japanese HD pedigrees. DNA was extracted from lymphocytes and lymphoblasts using the method of Kunkel *et al.* (1977). DNA (5 μ g) from these cells was digested with appropriate restriction endonucleases (*AccI*, *MboI*, *PstI*, or *TaqI*), fractionated on 0.8–1.0% agarose gels and transferred to Gene-Screen (Du Pont, NEN Research) in an alkaline solution (0.5 N NaOH, 1.5 N NaCl). Southern blots were prehybridized in a hybridization solution containing 200 μ g/ml of denatured salmon sperm DNA, and hybridized at 65°C overnight in the same buffer with probes radioactively labeled by random priming (Feinberg and Vogel-

stein, 1983) ("random primer DNA labeling kit," Takara Shuzo), as described previously (Nguyen *et al.*, 1987). The filters were washed with $0.5 \times \text{SSC}$, 1% SDS or $0.2 \times \text{SSC}$, 1% SDS at $55\text{--}60^\circ\text{C}$ prior to autoradiography at -70°C for 1–5 days using X-ray film (X-OMAT, Kodak) with intensifying screen.

Linkage analysis. Two-point linkage analysis was performed using the LI-PED program, version 3 (Ott, 1976; Hodge *et al.*, 1979), which incorporates age correction. Autosomal dominant inheritance of a rare gene (frequency 0.0001%) (Kanazawa, 1982) was assumed, and final penetrance was estimated at 1.00 in our families. Parameters for the age correction were based on age-at-onset data on affected individuals in the Japanese population (Kanazawa, 1982). Allele frequencies for the various markers used were estimated by counting alleles in the married-in-individuals in our pedigrees.

RESULTS

RFLPs

As summarized in Table 1, the 1 kb probe, BS674, defining the genetic locus D4S95 (Wasmuth *et al.*, 1988), detected three separate polymorphic sites using three restriction endonucleases (*AccI*, *MboI*, and *TaqI*). PIC values for *AccI*, *MboI*, and *TaqI* polymorphisms are 0.22, 0.24, and 0.31, respectively. VNTR-like RFLPs were found when probes from cosmids overlapping two *NotI* linking clones

Table 1. RFLPs at D4S95, D4S115, and D4S111.

Locus	Probe name	Restriction enzyme	Allelic fragment (kb)	Allele frequency tested	Polymorphic information content
D4S95	BS674	<i>AccI</i>	6.8	0.86	0.22
			1.5	0.14	
		<i>MboI</i>	1.3	0.83	0.24
			0.7+0.6	0.17	
		<i>TaqI</i>	2.3	0.74	0.31
D4S115	p152.3	<i>PstI</i>	1.7	0.26	0.77
			3.30	0.14	
			2.55	0.11	
			2.44	0.14	
			2.41	0.33	
			2.38	0.25	
D4S111	p157.9	<i>PstI</i>	2.32	0.03	0.65
			2.20	0.39	
			2.11	0.36	
			2.01	0.25

Fig. 2. Simultaneous hybridization with two probes (D4S115 and D4S111) detecting multi-allele RFLPs near the Huntington disease gene and a probe detecting lambda DNA. The potential for simultaneous detection of the RFLPs was tested by hybridization to *Pst*I-digested DNAs from reference families. P and M indicate the father and mother of each reference family respectively. Lane 1 contains *Eco*T141-digested lambda DNA.

were used. These probes, p157.9 and p252.3, define the loci D4S111 and D4S115 (Pohl *et al.*, 1988; Bucan *et al.*, 1990; Poustka *et al.*, 1987), respectively, which are located 250 kb apart, and are both distal to D4S95. As shown in Fig. 2, these two multi-allele RFLPs were able to be visualized using *Pst*I simultaneously. As these polymorphism possesses high PIC values (0.77 for D4S115 and 0.65 for D4S111) larger than 0.5, they are considered to be highly informative (Botstein *et al.*, 1980).

Pairwise analysis

Segregation of the DNA markers was analyzed by the computer program LIPED (Ott, 1976; Hodge *et al.*, 1979) to calculate the likelihood of linkage for various values of θ , the recombination fraction, relative to the likelihood of non-linkage ($\theta=0.5$). Table 2 presents the results of these calculations as a lod score (z , $\log_{10}$ of the likelihood ratio) for each value of θ . Significant linkage was detected for the marker D4S95. Five families gave informative results for linkage with probe p674 (D4S95). It is tightly linked to HD, giving a maximum likelihood estimate of $\theta=0.00$ ($Z_{\max}=3.31$). We previously reported the tight linkage between D4S43 and HD ($Z_{\max}=3.348$ for $\theta=0.00$) (Kanazawa *et al.*, 1990). These two

Table 2. Pairwise lod scores for linkage of 4p16.3 makers to HD.

Locus 1	Locus 2	Recombination fraction (θ)						Maximum lod score	
		0.00	0.01	0.05	0.10	0.20	0.25	$Z_{\max}$	$\theta_{\max}$
HD	D4S95	3.31	3.27	3.09	2.79	2.01	n.d.	3.31	0.00
HD	D4S115	$-\infty$	0.07	1.55	1.91	1.67	1.37	1.92	0.12
HD	D4S111	$-\infty$	-2.52	-0.67	0.01	0.34	0.31	0.34	0.20
D4S95	D4S115	-198	0.14	1.27	1.51	1.24	0.96	1.51	0.11
D4S95	D4S111	n.d.	1.24	2.34	2.44	n.d.	n.d.	2.46	0.08
D4S115	D4S111	n.d.	n.d.	n.d.	4.06	3.42	2.77	4.07	0.11

not determined.

loci (D4S95 and D4S43) were mapped to the same segment in the physical map of this region (Bucan *et al.*, 1990). There was significant linkage between these two loci in our pedigrees, too ($Z_{\max}=4.12$ at $\theta_{\max}=0.03$).

There was no significant linkage between D4S115 and HD. The maximum lod score was 1.92 at $\theta=0.12$. A two point linkage analysis between the marker D4S111 and the HD gene yielded a lod score -2.055 for $\theta=0.015$.

DISCUSSION

Recombination events with HD have not decisively positioned the HD gene but suggest two non-overlapping locations (MacDonald *et al.*, 1989b). Three events indicate a location close to telomere, distal to all known RFLP markers (MacDonald *et al.*, 1989b; Robbins *et al.*, 1989) (The distal candidate location). In contrast, the more proximal location is defined at its distal boundary by a discrete recombination event occurring between two markers *i.e.*, D4S113 and D4S168 (MacDonald *et al.*, 1989b; Whaley *et al.*, 1991) (The internal candidate location). The proximal genetic limit to the location of HD was defined (Bates *et al.*, 1991). They found the critical recombination event occurring between D4S10 and D4S125. Extending from a crossover event occurring between D4S10 and D4S125 to one between D4S113 and D4S168, the internal candidate location spans as much as 2.5 Mb. The distal boundary of this region has recently been redefined by a crossover event occurring between D4S43 and D4S98 (Snell *et al.*, 1992), thereby reducing the maximal size of the region from 2.5 to 2.2 Mb. The internal location is also supported by the detection of a linkage disequilibrium *i.e.*, the non-random association of marker alleles with disease mutation, between HD gene and some RFLPs at the D4S95 and D4S98 loci (Snell *et al.*, 1989; Theilmann *et al.*, 1989; Adam *et al.*, 1991; MacDonald *et al.*, 1991, 1992). In addition, a new gene, IT15, isolated from the proxymal candidate location contains a polymorphic trinucleotide repeat that is expanded on HD chromosomes (The Huntington Disease Collaborative Research Group, 1993).

In the present study, a proximal marker D4S95 showed tight linkage to the disease locus in Japanese pedigrees, while distal markers D4S115 and D4S111 did not. As shown in Fig. 1, D4S95 is located in the proximal candidate location, while both D4S115 and D4S111 are distal to it. There was a gradual loss of positive lod scores as we go through proximal to distal. Particularly, a two point linkage analysis between D4S111 and HD yielded a lod score -2.01 for $\theta=0.015$. As a lod score of ≤ -2 is used in determining whether exclusionary evidence is obtained (Morton, 1955), our result lead to the exclusion, as a possible region of localization of the HD gene, of more than 3 cM of the genome around D4S111 locus. At the same time, our results favor aforementioned proximal location as a candidate location for the HD gene.

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