1983; 94.4, 2.8, 1.1, and 1.7 in 1984; and 94.4, 2.9, 1.1, and 1.6 in 1985. We assumed the percentages before 1977 varied linearly from their 1951 values (100, 0, 0, and 0) to their 1977 values.
19. Specifically, we assumed that $a = 1/24 \pm 1/24$ after 1964 and $0 \pm 1/24$ before 1964, $b = 1/12 \pm 1/12$, and $f = 0.94 \pm 0.02$, based on industry evaluations (*1*). Uncertainties are 2σ.
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A DNA Segment Encoding Two Genes Very Tightly Linked to Huntington's Disease

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The discovery of D4S10, an anonymous DNA marker genetically linked to Huntington's disease (HD), introduced the capacity for limited presymptomatic diagnosis in this late-onset neurodegenerative disorder and raised the hope of cloning and characterizing the defect based on its chromosomal location. Progress on both fronts has been limited by the absence of additional DNA markers closer to the HD gene. An anonymous DNA locus, D4S43, has now been found that shows extremely tight linkage to *HD*. Like the disease gene, D4S43 is located in the most distal region of the chromosome 4 short arm, flanked by D4S10 and the telomere. In three extended HD kindreds, D4S43 displays no recombination with HD, placing it within 0 to 1.5 centimorgans of the genetic defect. Expansion of the D4S43 region to include 108 kilobases of cloned DNA has allowed identification of eight restriction fragment length polymorphisms and at least two independent coding segments. In the absence of crossovers, these genes must be considered candidates for the site of the HD defect, although the D4S43 restriction fragment length polymorphisms do not display linkage disequilibrium with the disease gene.

HUNTINGTON'S DISEASE (HD), a neurodegenerative disorder of mid-life onset, is caused by a highly penetrant dominant defect that causes specific neuronal loss, leading to progressive motor disturbance, psychological manifestations, and intellectual deterioration (*1*). No treatment is effective in halting or delaying the inexorable progression of HD, which results in complete disability, and ultimately death, typically 15 to 20 years after onset.

The biochemical basis for the cell death in HD is not understood. Recent efforts to elucidate the nature of the genetic defect have concentrated on the chromosomal position of the HD gene, located 4 centimorgans (cM) from the anonymous DNA marker D4S10 in the terminal cytogenetic band of the chromosome 4 short arm (*2*). The recombination rate implies a physical distance of several million base pairs between the two loci, a strong deterrent to the application of chromosome walking strategies for cloning the HD gene. Furthermore, this genetic separation, together with the absence of a marker flanking the disease locus, have limited the accuracy which can be achieved in presymptomatic diagnosis of this disorder.

D4S10 has been physically mapped to 4p16.3, the terminal cytogenetic subband, which comprises about 3% of the cytogenetic length of chromosome 4 (*3*). Multipoint linkage analysis with additional proximal DNA markers has established that the HD gene is located closer to the telomere than D4S10, which limits the potential for isolating probes closer to or flanking the HD gene (*2*). By physically mapping more than 200 randomly chosen chromosome 4 probes, we have identified a single locus, D4S43, that has the potential for being closer to, or flanking the HD defect (*2, 3*).

The D4S43 locus was initially defined by the anonymous DNA probe C4H, which is a 3.0-kb single-copy Hind III fragment derived from a chromosome 4–specific library (*2, 3*). C4H detects two relatively infrequent restriction fragment length polymorphisms (RFLPs) in Bcl I– or Msp I–digested DNA (Table 1) which were uninformative for linkage in most HD kindreds, including the extended Venezuela HD pedigree critical to the discovery of the D4S10 marker (*2*). However, these RFLPs were segregating in a portion of the Venezuela family used as a "reference" pedigree for determining the linkage relationships of DNA markers (*4*). D4S43 was linked to D4S10 with a maximum logarithm of the odds (lod) score of $\hat{z} = 31.97$ at a recombination fraction $\hat{\theta} = 0.04$ (Table 2), but showed very loose linkage to *RAF2*, a proximal marker located in 4p16.1. Multipoint analysis (*5*) of the three loci revealed that a position of D4S43 distal to D4S10 was favored by more than 10^5:1 relative to a location between the other two markers, or proximal to *RAF2*. D4S43 therefore maps at the same approximate genetic distance from D4S10 as *HD*, in the terminal segment of the short arm of chromosome 4 bordered by D4S10 and the telomere (*2*).

To find more informative RFLPs at the D4S43 locus, we isolated a cosmid clone, C9A, which contains the C4H segment. Figure 1 includes a restriction map of C9A, with the locations of three single-copy fragments C4H, HB1.4, and H2.0. The integrity of the C9A cosmid was tested by mapping each of these fragments to the same terminal region of 4p by means of a somatic cell hybrid panel (*3*). This analysis revealed that HB1.4 detected significant homology in rodent DNAs, suggesting that it contained a conserved coding sequence. Consequently we probed a human liver complementary DNA (cDNA) library to obtain LCD, a cDNA clone encoded within a 13-kb stretch of the D4S43 region (Fig. 1).

Three additional infrequent RFLPs were identified with H2.0 or portions of LCD, but these only modestly increased the general utility of the locus (Table 1). However,

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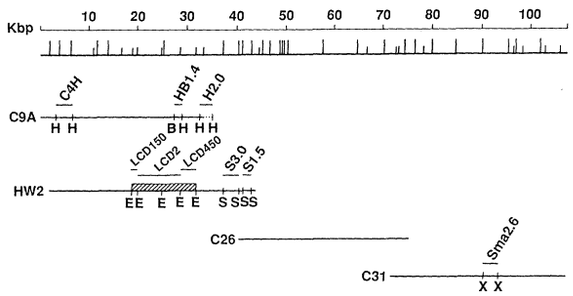

Fig. 1. Overlapping cosmid clones for the D4S43 locus showing the positions of the single-copy probes used for detecting RFLPs. Overlapping pCOS2EMBL cosmid clones spanning 108 kb were obtained by sequential chromosome walking unidirectionally from C4H (*10*). Attempts to walk in the opposite direction did not produce any cosmid clones extending further than C9A and HW2. The long and short vertical lines shown over the cloned region represent Sac I and Eco RI sites, respectively. These were determined with oligonucleotide probes specific for the λcos ends in conjunction with partial digestion of cosmid DNA linearized with λ terminase (*11*). Additional restriction enzyme sites denoting subcloned fragments are shown beneath the individual cosmid inserts as follows: H = Hind III, B = Bgl II, E = Eco RI, S = Sac I, and X = Sma I. C4H, HB1.4, H2.0 (which contains some vector sequence denoted by the dotted line), S3.0, and S1.5 are all free of repetitive DNA elements. The Sma2.6 probe has a slight amount of repetitive DNA and must therefore be hybridized to Southern blots only after first preannealing with unlabeled human genomic DNA to reduce signal from repeat sequences (*12*). HB1.4 was used to isolate a cDNA clone, LCD, from a human liver cDNA library constructed in λgt11. This cDNA contained three Eco RI fragments of 1.2 kb (LCD2), 0.45 kb (LCD450), and 0.15 kb (LCD150) which derive from the hatched area of HW2. These single-copy cDNA fragments were superior for RFLP screening to genomic fragments from the same area that contained repetitive elements. The S1.5 probe detected a second independent coding sequence for which cDNAs were isolated, but these did not reveal additional RFLPs, as did several single-copy fragments from C26.

Table 1. RFLPs for the D4S43 locus.

Probe		Restriction enzyme	Invariant fragments (kb)	Allelic fragments (kb)	Frequency	*N*†	PIC
Name	Fragment						
C4H	3.0 kb Hind III	Msp I		7.9	0.92	180	0.14
				5.5 + 2.4	0.08		
		Bcl I	5.6	4.4	0.90	186	0.16
				3.3	0.10		
LCD 150	0.15 kb Eco RI	Sau 96I		4.8	0.94	148	0.11
				3.2	0.06		
LCD 2	1.2 kb Eco RI	Sau 96I	1.9*	4.9	0.994	164	0.01
				3.6 + 1.3	0.006		
H2.0	2.0 kb Hind III	Bam HI		7.5	0.97	116	0.06
				7.0	0.03		
S3.0	3.0 kb Sac I	Taq I	*	2.7	0.04	48	0.07
				2.6	0.96		
S1.5	1.5 kb Sac I	Taq I		3.4	0.19	118	0.26
				2.3	0.81		
Sma2.6	2.6 kb Sma I	Msp I + others		2.3	0.35	134	0.58
				2.2	0.22		
				2.1	0.01		
				2.0	0.42		

*LCD2 and S3.0 also detect the allelic fragments for the LCD150 and S1.5 RFLPs, respectively, as less intensely hybridizing bands that are difficult to visualize reproducibly. †*N*, number of chromosomes typed.

the rare allele of a Sau 96I RFLP (present on only 0.6% of normal chromosomes) cosegregated with HD in the extended Venezuelan pedigree, permitting an accurate assessment of the proximity of D4S43 to the genetic defect. Table 2 presents the lod scores obtained for linkage of the D4S43 locus to HD in the Venezuelan pedigree, and in two other large pedigrees for which the C4H RFLPs were informative (*6*). The peak lod score, $\hat{z} = 44.75$, occurs at a recombination fraction $\hat{\theta} = 0.00$, indicating that no recombination events were seen between D4S43 and *HD* in any of the families. The 1-lod unit confidence interval extends from $\theta = 0.00$ to 0.015, establishing that D4S43 is much closer to *HD* than D4S10. Without a crossover between the two loci, it is not possible to determine whether D4S43 is distal to *HD*, and therefore flanking the disease locus relative to D4S10.

When informative in predictive testing, D4S43 will provide much greater accuracy than D4S10. Consequently, we have continued to increase the overall level of heterozygosity of the new locus. Figure 1 shows three cosmids (HW2, C26, and C31) that represent sequences adjacent to C9A. The cosmids contain fragments S3.0, S1.5, and Sma2.6, which were used as probes to identify additional RFLPs. The S1.5 segment hybridizes to a discrete fragment in rodent DNA, and represents a second expressed sequence, independent from the LCD segment. S1.5 and S3.0 both detect separate Taq I RFLPs (Table 1), but the informativeness of the D4S43 locus is dramatically enhanced by a multi-allele system detected by the Sma2.6 probe. By analogy to the VNTR markers described recently by Nakamura *et al.* (*7*), the basis for the Sma2.6 RFLP may be a variable number of small tandem repeats. Although we have described four alleles for this marker system, we anticipate that under optimal electrophoresis conditions it may be possible to resolve additional allelic fragments. The polymor-

Table 2. Lod scores for linkage of D4S43 with D4S10, *RAF2*, and *HD*.

Pedigree	Locus 1	Locus 2	Recombination fraction (θ)							Maximum lod score ($\hat{z}$)	Confidence interval (1-lod unit)
			0.00	0.01	0.05	0.10	0.20	0.30	0.40		
Venezuela reference	D4S43	*RAF2*	$-\infty$	−25.86	−9.71	−2.76	2.13	2.95	1.80	2.98 ($\hat{\theta} = 0.28$)	0.19–0.38
Venezuela reference	D4S43	D4S10	$-\infty$	29.88	31.93	30.31	24.42	16.96	8.18	31.97 ($\hat{\theta} = 0.04$)	0.02–0.09
Venezuela HD fam.	*HD*	D4S43	37.62	37.05	34.69	31.55	24.64	16.82	8.04		
American HD fam. 1	*HD*	D4S43	1.85	1.80	1.58	1.30	0.72	0.17	0.00		
American HD fam. 2	*HD*	D4S43	5.28	5.21	4.88	4.40	3.29	2.03	0.75		
Total HD pedigrees	*HD*	D4S43	44.75	44.06	41.15	37.25	28.65	19.02	8.79	44.75 ($\hat{\theta} = 0.00$)	0.00–0.015

phism information content (PIC) (*8*) of the Sma2.6 RFLP is 0.58, raising the overall PIC of the D4S43 locus to an estimated 0.70. Thus, the marker should be informative in the majority of families for which presymptomatic diagnosis is attempted.

The predictive value of the D4S43 locus would be further enhanced if any of the RFLPs displayed a strong association with the disease gene due to linkage disequilibrium between the two loci. In this instance, presymptomatic diagnosis could potentially be extended to those "at risk" individuals who have too few living relatives for application of the linkage test. Such a result would also suggest that D4S43 and *HD* are in extremely close proximity. To test for linkage disequilibrium, we typed a collection of unrelated individuals affected by HD (*9*), along with their respective unaffected, and sometimes affected parents. The unaffected parents provide a control group for determining the frequency of individual alleles on normal chromosomes. Comparison of the parental genotypes with those of the affected offspring served to identify in most instances the particular allele inherited together with the HD gene in a given family. For all eight RFLPs, we observed no significant association between the defect and any specific allele ($P > 0.05$ in all cases). Furthermore, when alleles for the individual RFLPs were considered together, a minimum of ten different haplotypes were present on HD chromosomes. Thus, either the assumption that most affected individuals have inherited an HD defect of common origin is incorrect, or the D4S43 marker is not close enough to the defect to detect linkage disequilibrium without examining individuals from specific ethnic groups or geographic locations.

The position of D4S43 makes it the closest available marker to the HD gene, increasing both the accuracy and applicability of predictive testing. The absence of crossovers with HD, together with a physical localization within the terminal 3% of the short arm of chromosome 4, make D4S43 a suitable starting point for progressing toward the HD gene by chromosome walking, long-range physical mapping by pulsed field gel electrophoresis, and sophisticated directional cloning techniques such as chromosome "jumping." Although it is impossible to estimate the exact physical distance that separates the two loci, both are bracketed by D4S10 and the telomere within a region probably containing about 0.2% of the genome (*2*). With no crossovers detected between D4S43 and *HD*, the two independent coding sequences represented by the LCD cDNA, and a different cDNA isolated by means of S1.5, remain candidates for the site of the HD defect.

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6. The three pedigrees included in the lod score analysis were known to contain a minimum of five recombinations between D4S10 and *HD* that were not detected by D4S43. In addition, individual recombinations between D4S10 and *HD* in five other families were similarly not detected by D4S43, but these pedigrees were not included in the computer analysis since only selected individuals had been typed.
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Long-Term Neuropathological and Neurochemical Effects of Nucleus Basalis Lesions in the Rat

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The long-term effects of excitotoxic lesions in the nucleus basalis magnocellularis of the rat were found to mimic several neuropathological and chemical changes associated with Alzheimer's disease. Neuritic plaque–like structures, neurofibrillary changes, and neuronal atrophy or loss were observed in the frontoparietal cortex, hippocampus, amygdala, and entorhinal cortex 14 months after the lesions were made. Cholinergic markers in neocortex were reduced, while catecholamine and indoleamine metabolism was largely unaffected at this time. Bilateral lesions of the nucleus basalis magnocellularis increased somatostatin and neuropeptide Y in the cortex of the rat by at least 138 and 284 percent, respectively, suggesting a functional interaction between cholinergic and peptidergic neurons that may differ from that in Alzheimer's disease.

ALZHEIMER'S DISEASE (AD), THE most common cause of dementia in the elderly, is consistently associated with a loss or dysfunction of cholinergic neurons in the nucleus basalis of Meynert (NBM) (*1, 2*) that project to the cerebral cortex. Other transmitters such as the peptides somatostatin (SS) and neuropeptide Y (NPY) are also reduced in the cortices of some brains from patients with AD (*3*), suggesting their involvement in the disease process. Neuropathologically, AD is characterized by cerebral atrophy due to substantial neuronal losses primarily in two brain regions important for cognitive functioning—the cerebral cortex and hippocampus (*4*). Within these regions of AD brains there are many neuritic plaques and neurofibrillary tangles (*5*).

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