

ted detail with, for example, maps of the atmospheric winds and of radiation anomalies. Despite this increased knowledge of the atmospheric changes, there is still a great deal that is unknown and unanswered, particularly in the ocean. Clearly it would be beneficial to be able to model the progress of warm events in the same way as we currently model the development of weather systems, but this will require greatly expanded observing networks in the tropical oceans.

Received 8 July; accepted 28 September 1983.

1. Philander, S. G. H. *Nature* **302**, 295–301 (1983).
2. Philander, S. G. H. *Nature* **305**, 16 (1983).
3. Rasmusson, E. M. & Carpenter, T. H. *Mon. Weath. Rev.* **110**, 354–384 (1982).
4. Walker, G. T. *Mem. Ind. met. Dept* **24**, 275–332 (1924).
5. Barber, R. T. & Chavez, F. *Science* (in the press).
6. Rasmusson, E. M. & Wallace, J. M. *Science* (in the press).
7. Cane, M. *Science* (in the press).
8. Shreiber, R. W. & Schreiber, E. A. *Trop. Ocean-Atmos. Newsltt.* **16**, 10–12 (1983).
9. Newell, R. E., Kidson, J. W., Vincent, D. G. & Boer, G. J. *The General Circulation of the Tropical Atmosphere* Vol 2 (MIT Press, Cambridge, 1974).
10. Gill, A. E. *Q. Jl R. met. Soc.* **106**, 447–462 (1980).

11. Heckley, W. A. & Gill, A. E. *Q. Jl R. met. Soc.* **40**, 1613–1630 (1983).
12. Lau N-C. *Mon. Weath. Rev.* **109**, 2287–2311 (1981).
13. Shukla, J. & Wallace, J. M. *J. Atmos. Sci.* **40**, 1613–1630 (1983).
14. Niiler, P. & Stevenson, J. *J. mar. Res. Suppl.* **40**, 465–480 (1982).
15. Gill, A. E. *J. phys. Oceanogr.* **13**, 586–606 (1982).
16. Gill, A. E. Models of equatorial currents. In *Numerical Models of Ocean Circulation*, 181–203 (National Academy of Science, Washington DC, 1975).
17. Hickey, B. *J. phys. Oceanogr.* **5**, 460–475 (1975).
18. Wyrtki, K. *J. phys. Oceanogr.* **5**, 572–584 (1975).
19. Barnett, T. P. *J. phys. Oceanogr.* **11**, 1043–1058 (1981).
20. McCreary, J. *J. phys. Oceanogr.* **6**, 632–645 (1976).
21. Busalacchi, A. J. & O'Brien, J. J. *J. geophys. Res.* **86**, 10901–10907 (1981).
22. Busalacchi, A. J., Takeuchi, K. & O'Brien, J. J. in *Hydrodynamics of the Equatorial Ocean* (ed. Nihoul, J. C. J.) 155–195 (Elsevier, Amsterdam, 1983).
23. Gill, A. E. *Atmosphere-Ocean Dynamics* (Academic, New York, 1982).
24. Lukas, R. & Firing, E. *Trop. Ocean-Atmos. Newsltt.* **16**, 9–10; **21**, 9–11 (1983).
25. Vitousek, M. *Trop. Ocean-Atmos. Newsltt.* **16**, 10 (1983).
26. Toole, J. M. *Trop. Ocean-Atmos. Newsltt.* **16**, 12–14 (1983).
27. Halpern, D., Hayes, S. P., Leetmaa, A., Hansen, D. V. & Philander, S. G. H. *Science* (in the press).
28. Leetmaa, A. *J. phys. Oceanogr.* **13**, 467–473 (1983).
29. Philander, S. G. H. & Pacanowski, R. C. *Tellus* **33**, 201–210 (1981).
30. Gill, A. E. *J. phys. Oceanogr.* **12**, 1372–1387 (1982).
31. Smith, R. L. & Huyer, A. *Trop. Ocean-Atmos. Newsltt.* **21**, 28–29 (1983).
32. Smith, R. L. *Science* (in the press).
33. Reynolds, R. W. *A Monthly Averaged Climatology of Sea Surface Temperature*, NOAA Tech. Rep. NWS **31** (1982).

A polymorphic DNA marker genetically linked to Huntington's disease

James F. Gusella*, Nancy S. Wexler[†], P. Michael Conneally[†], Susan L. Naylor[§], Mary Anne Anderson*, Rudolph E. Tanzi*, Paul C. Watkins[¶], Kathleen Ottina*, Margaret R. Wallace[‡], Alan Y. Sakaguchi[§], Anne B. Young^{||}, Ira Shoulson^{||}, Ernesto Bonilla^{||} & Joseph B. Martin*

* Neurology Department and Genetics Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114, USA

[†] Hereditary Disease Foundation, 9701 Wilshire Blvd, Beverly Hills, California 90212, USA

[‡] Department of Medical Genetics, Indiana University Medical Center, Indianapolis, Indiana 46223, USA

[§] Department of Human Genetics, Roswell Park Memorial Institute, Buffalo, New York 14263, USA

^{||} Venezuela Collaborative Huntington's Disease Project*

Family studies show that the Huntington's disease gene is linked to a polymorphic DNA marker that maps to human chromosome 4. The chromosomal localization of the Huntington's disease gene is the first step in using recombinant DNA technology to identify the primary genetic defect in this disorder.

HUNTINGTON'S disease (also known as Huntington's chorea) is a progressive neurodegenerative disorder with autosomal dominant inheritance¹. The first symptoms of Huntington's disease usually occur in the third to fifth decade and the gene is completely penetrant. The disease is characterized by both progressive motor abnormality, typically chorea, and intellectual deterioration commonly accompanied by prominent psychiatric symptoms including severe depression. The symptoms of Huntington's disease result from premature neuronal cell death which is most marked in the basal ganglia. Although the prevalence of Huntington's disease is only 5–10 in 100,000, because of its late onset most individuals have children before they realize they have inherited Huntington's disease, and it thus significantly affects a much greater proportion of the population². In spite of numerous biochemical studies of peripheral tissues and of post-mortem brain tissue the primary defect in Huntington's disease has never been detected^{3,4}. At present there is no reliable method of presymptomatic or prenatal diagnosis of the disease and there is no effective therapy.

A number of investigators have examined Huntington's disease pedigrees in search of a genetic marker linked to the

Huntington's disease locus^{5,6}. Such studies have proved difficult because of the late age of onset of the disorder and the consequent need for a large number of individuals to be typed. Although investigations relying on classical polymorphic antigen and enzyme markers were uniformly negative, they did exclude the Huntington's disease locus from 20% of the human genome⁵. The lack of additional polymorphic protein markers precluded the possibility of testing remaining regions of the genome.

In recent years, the techniques for circumventing this difficulty have been developed^{7,8}. Recombinant DNA technology provides a method for obtaining the requisite number of new genetic markers because it permits the monitoring of heritable differences in the sequence of genomic DNA. These DNA markers are termed restriction fragment length polymorphisms (RFLPs). They are detected as differences in the sizes of restriction fragments observed in Southern blotting experiments on human genomic DNA using cloned DNA probes free of repetitive sequences. Unlike classical expressed markers, DNA polymorphisms can be found in regions of the genome irrespective of whether they encode a protein. Probes that detect RFLPs can therefore be derived from known gene loci or from anonymous DNA segments. RFLPs appear to be present in all regions of the genome, thus making it feasible to construct a detailed human genetic linkage map and thereby localize disease genes^{7–11}. We have now identified an anonymous DNA fragment from human chromosome 4 that detects two different RFLPs in a *Hind*III digest of human genomic DNA. This polymorphic DNA marker shows close genetic linkage to the Huntington's

† Present address: Integrated Genetics Inc., 51 New York Avenue, Framingham, Massachusetts 01701, USA.

* Members of the Venezuela Collaborative HD Project included: R. Erbe, E. Bonilla, I. de Quiroz, J. Esteves, H. Moreno, K. Villa Lobos, S. Bailey, F. A. Burnham, M. Dorn, E. Goldstein, A. Greene, F. Gomez, K. Kidd, J. Kidd, E. Messer, Z. Layrisse, N. Marsol, J. Penny, A. Young, G. H. Rosenzweig, I. Shoulson, S. Starosta, R. Snodgrass, H. Travers, M. Rivas, R. S. Uzzell and A. R. Wexler.

disease gene in two separate families, although a different haplotype of the marker segregates with the Huntington's disease gene in each family. We infer that the Huntington's disease locus resides on human chromosome 4.

Huntington's disease families

The success of any genetic linkage project depends in large part on the quality of family material available for study. We initially invested considerable effort in identifying a useful family from the National Research Roster for Huntington's Disease Patients and Families at Indiana University. An American family of reasonable size was selected (Fig. 1) and blood samples were obtained to establish permanent Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines. Subsequently, a substantially larger Huntington's disease family was located (Fig. 2)¹². This pedigree stems from a unique community of interrelated Huntington's disease gene carriers living along the shores of Lake Maracaibo, Venezuela. In a pedigree numbering over 3,000 since the early 1800s, all Huntington's disease patients have inherited the defective gene from a common ancestor. This is the largest known concentration of Huntington's disease in one family. For the past three years, an expedition has spent one month annually in Venezuela collecting pedigree information, tissue samples, and clinical data (N. S. Wexler *et al.*, manuscript in preparation). Permanent lymphoblastoid cell lines were again established for each individual to act as a permanent source of genomic DNA. In both families, each individual was examined by at least one neurologist experienced and knowledgeable about Huntington's disease. In Venezuela, many family members were examined for three consecutive years. Individuals were diagnosed as having Huntington's disease on the basis of family history, abnormal motor function and intellectual impairment. Each was assigned to a functional stage reflecting physical and social functioning according to a scale developed by Shoulson and Fahn¹³. Blood samples from members of both pedigrees were analysed using at least 20 red cell and plasma markers to exclude cases of non-paternity.

Characterization of bacteriophage clone G8

A number of DNA probes that detect RFLPs have been identified in this and other laboratories. Some of these probes represent DNA sequences from known gene loci whilst others are anonymous DNA segments chosen because they contain no repetitive DNA sequences. In an initial screen for linkage to the Huntington's disease gene, 12 such DNA markers were tested in the American family depicted in Fig. 1. Of these markers only one, G8, gave a suggestion of linkage to the Huntington's disease gene¹⁴.

The G8 clone is a recombinant bacteriophage from the human gene library of Maniatis and co-workers¹⁵. It contains 17.6 kilobases (kb) of human DNA free of repetitive sequences. In our previous studies, G8 DNA was used as a probe in Southern blotting experiments in an attempt to identify RFLPs¹⁶. The probe detected two invariant and several variable *Hind*III fragments in human genomic DNA. The pattern of hybridization of G8 to *Hind*III-digested DNAs from members of two families is shown in Fig. 3. The invariant *Hind*III fragments are seen at 8.4 kb and 2.3 kb. Other fragments whose presence varied from individual to individual (in these and other unrelated individuals not shown in the figure) are seen at 17.5 kb, 15 kb, 4.9 kb, 3.7 kb and 1.2 kb. In order to determine the basis for this variation, we constructed a restriction map of clone G8.

In our initial attempts to derive this map, we determined that the G8 insert contained five *Eco*RI fragments of 6.0 kb, 5.5 kb, 2.4 kb, 2.2 kb and 1.5 kb. We subcloned each of these *Eco*RI fragments into a plasmid vector (pBR322 or pUC9). *Hind*III sites within each *Eco*RI fragment were mapped by direct digestion of the subcloned DNA with *Hind*III. The order of the *Eco*RI fragments within the bacteriophage clone was determined by using each subclone as a probe against single or double *Hind*III and *Eco*RI digests of G8 DNA.

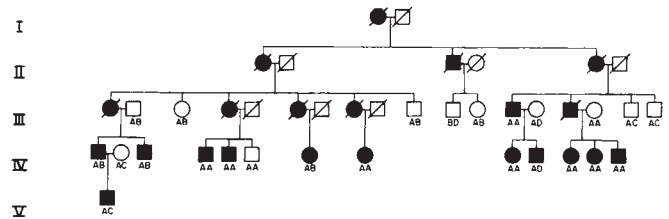


Fig. 1 Pedigree of an American Huntington's disease family. Symbols: circles, females; squares, males; a black symbol indicates that an individual is affected with Huntington's disease; a slashed symbol indicates that an individual is deceased. This pedigree was identified through the National Research Roster for Huntington's Disease Patients and Families at Indiana University. Relevant family members were examined by a neurologist and blood samples were obtained. EBV-transformed lymphoblastoid cell lines were established for the individuals whose genotypes are shown and have been stored at the Human Genetic Mutant Cell Repository, Camden, New Jersey. Phenotypes at the G8 locus shown under each symbol were determined by Southern blotting as outlined in Fig. 3. For the purposes of confidentiality, selected individuals are not shown.

Variation at one *Hind*III site, called site 1, occurs outside of the actual cloned sequence in G8. It is detected in Southern blot experiments by hybridization to sequences at the left end of the map shown in Fig. 4. When this *Hind*III site is present, a 15 kb fragment is seen; when it is absent a 17.5 kb fragment is seen. The second *Hind*III RFLP results from the presence or absence of a site, called site 2, within the G8 insert. When this site is present, two fragments are seen, 3.7 kb and 1.2 kb, but in its absence, only a single fragment of 4.9 kb is detected.

A survey of 23 unrelated North Americans gave allele frequencies for *Hind*III site 1, occurring outside the clone, of 0.74 for the absence of the site, and 0.26 for the presence of the site. For *Hind*III site 2, mapping within the G8 clone, allele frequencies were 0.81 for the presence of the site and 0.19 for the absence of the site. In each case we have termed the more frequent allele 1, and the minor allele 2 (see Fig. 3). Alterations in restriction fragment length due to the presence or absence of each of these sites display a Mendelian pattern of inheritance. Each *Eco*RI subclone was also used as a probe against *Hind*III digests of genomic DNA. The restriction map of the insert in G8 and the corresponding genomic region including the positions of the two variable *Hind*III sites is shown in Fig. 4.

When polymorphic sites in the DNA map are in such close proximity that the frequency of recombination between them is negligible, they are inherited together as a unit. The combined information from both sites on a given chromosome can then be considered as a single haplotype. At the G8 locus, the two polymorphic *Hind*III sites are separated by only 18.7 kb. Family studies were carried out using members of the Venezuela pedigree heterozygous at both sites to test for co-segregation of the two RFLPs. Because of the predictive nature of the data, the phenotypes observed for these 'at risk' individuals are not shown to protect their confidentiality. The data were analysed using the computer program LIPED¹⁷. A lod score of 4.53 was obtained at a recombination fraction (θ) of 0.0. No crossovers were seen between the two sites, supporting the hypothesis that the RFLPs are inherited together as a haplotype. The four possible combinations of alleles at site 1 and site 2 have been termed haplotypes A, B, C and D as explained in Fig. 4.

If no linkage disequilibrium exists between the two polymorphic *Hind*III sites then the frequency of these haplotypes in the population will be 0.61, 0.14, 0.20, and 0.05 respectively. These frequencies are predicted from the allele frequencies at each polymorphic *Hind*III site. In our limited sample of 23 individuals, we observed frequencies for A, B, C and D of 0.64, 0.11, 0.22 and 0.04 indicating that there is not a high degree of disequilibrium in the general North American population between these two RFLPs. A much larger sample of unrelated individuals will be necessary to determine with certainty whether

Fig. 2 Pedigree of the Venezuelan Huntington's disease family. This pedigree represents a small part of a much larger pedigree that will be described in detail elsewhere. Permanent EBV-transformed lymphoblastoid cell lines were established from blood samples of these individuals (unpublished data). DNA prepared from the lymphoblastoid lines will be used to determine the phenotype of each individual at the G8 locus as described in Fig. 3. The data were analysed for linkage to the Huntington's disease gene using the program LIPED¹⁷ with a correction for the late age of onset⁵. Because of the high frequency of the Huntington's disease gene in this population some of the spouses of affected individuals have also descended from identified Huntington's disease gene carriers. In none of these cases, however, was the unaffected individual at significantly greater risk for Huntington's disease than a member of the general population. Although a number of younger at-risk individuals were also analysed as part of this study, for the sake of these family members the data are not shown due to their predictive nature. The data are available upon request if confidentiality can be assured.

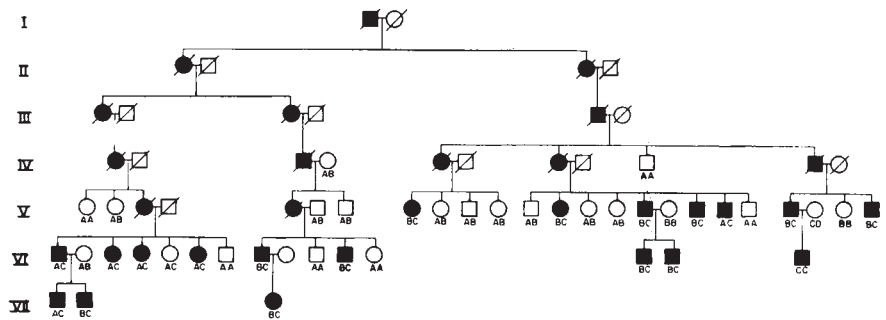
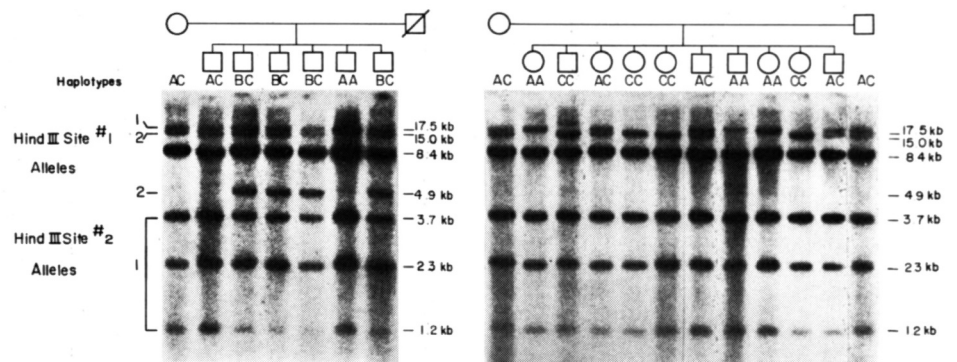


Fig. 3 Hybridization of the G8 Probe to *Hind*III-digested human genomic DNA.

Methods: DNA was prepared as described²³ from lymphoblastoid cell lines derived from members of two nuclear families. 5 μ g of each DNA was digested to completion with 20 units of *Hind*III in a volume of 30 μ l using the buffer recommended by the supplier. The DNAs were fractionated on a 1% horizontal agarose gel in TBE buffer (89 mM Tris, pH 8, 89 mM Na borate, 2 mM Na EDTA) for 18 h. *Hind*III-digested λ C1857 DNA was loaded in a separate lane as a size marker.

The gels were stained with ethidium bromide (0.5 μ g ml⁻¹) for 30 min and the DNA was visualized with UV light. The gels were incubated for 45 min in 1 M NaOH with gentle shaking and for two successive 20 min periods in 1 M Tris, pH 7.6, 1.5 M NaCl. DNA from the gel was transferred in 20 \times SSC (3 M NaCl, 0.3 M Na citrate) by capillary action to a positively charged nylon membrane. After overnight transfer, agarose clinging to the filters was removed by washing in 3 \times SSC and the filters were air dried and baked for 2 h under vacuum at 80 $^{\circ}$ C. Baked filters were prehybridized in 500 ml 6 \times SSC, 1 \times Denhardt's solution (0.02% bovine serum albumin, 0.02% polyvinyl pyrrolidone, 0.02% Ficoll), 0.3% SDS and 100 μ g ml⁻¹ denatured salmon sperm DNA at 65 $^{\circ}$ C for 18 h. Prehybridized filters were washed extensively at room temperature in 3 \times SSC until no evidence of SDS remained. Excess liquid was removed from the filters by blotting on Whatman 3MM paper and damp filters were placed individually in heat-sealable plastic bags. 5 ml of hybridization solution (6 \times SSC, 1 \times Denhardt's solution, 0.1% SDS, 100 μ g ml⁻¹ denatured salmon sperm DNA) containing approximately 5 \times 10⁶ c.p.m. of nick-translated G8 DNA (specific activity \sim 2 \times 10⁸ c.p.m. μ g⁻¹)²⁴ was added to each bag which was then sealed and placed at 65 $^{\circ}$ C for 24–48 h. Filters were removed from the bags and washed at 65 $^{\circ}$ C for 30 min each in 3 \times SSC, 2 \times SSC, 1 \times SSC and 0.3 \times SSC. The filters were dried and exposed to X-ray film (Kodak XR-5) at -70 $^{\circ}$ C with a Dupont Cronex intensifying screen for 1 to 4 days. The haplotypes observed in each individual were determined from the alleles seen for each *Hind*III RFLP (site 1 and 2) as explained in Fig. 4.



any significant degree of disequilibrium exists. The predicted level of heterozygosity at the G8 locus if the two sites are in equilibrium is 57%, making this an excellent genetic marker.

Mapping of G8 to chromosome 4

The G8 sequence was mapped to a human chromosome by Southern blot analysis of human-mouse somatic cell hybrids¹⁸. The presence or absence of fragments detected by the G8 probe was determined for 18 karyotyped hybrids (Table 1). These fragments were always seen when chromosome 4 was present in the hybrid cell line, but never seen when chromosome 4 was absent. For all other chromosomes there were several discordant clones. Seven of the hybrids were tested using the enzyme *Hind*III, whilst the rest were monitored after *Eco*RI digestion. In both cases, all fragments detected in total genomic DNA co-segregated with chromosome 4.

An additional 28 hybrids were tested only for enzyme markers previously assigned to each of the human chromosomes. Two discordant clones were seen with the chromosome 4 marker peptidase S, probably due to the relative insensitivity of the peptidase S assay compared to Southern blot hybridization. A much higher rate of discordance (>26%) was seen for all other human chromosomes. These data clearly assign the fragments detected by G8 to chromosome 4. The G8 DNA segment was recently given the designation D4S10 at The Human Gene Mapping Workshop 7¹⁹.

Analysis of linkage

After initial data were obtained for American Huntington's disease family, selected members of the Venezuela family were typed using marker G8. The haplotypes observed in these families are displayed in Figs 1 and 2. The data were analysed for linkage of the DNA marker to the Huntington's disease gene using the program LIPED¹⁷. At-risk individuals included in the analysis were assigned a probability of having inherited the gene based on an age of onset function as previously described.⁵ The cumulative distribution of age of onset was assumed to be similar in the American and Venezuelan families.⁵ Haplotype frequencies used for the G8 marker were those predicted from the individual allele frequencies for the site 1 and site 2 RFLPs assuming complete equilibrium. It should be noted that these frequencies were observed in the North American population and their exact values in the Venezuela population might vary from these estimates. Results of the analysis for G8 and Huntington's disease, together with those for two other chromosome 4 markers are given in Table 2.

The maximum lod score for Huntington's disease against G8 was 8.53 at a θ of 0.00, suggesting that the two loci are very closely linked. The maximum lod score was obtained at a θ of 0.00 for both sexes. No recombinants can be detected in either pedigree. The Huntington's disease gene segregated with the A haplotype of G8 in the American family and with the C in the Venezuela pedigree.

Table 1 Segregation of G-8 with human chromosomes in human-mouse hybrids

Cell hybrid	Chromosomes																						Translocated chromosomes		
	G-8	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		22	X
WIL-5	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	+	
WIL-2	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	+	-	+	
WIL-7	-	-	+	+	-	+	+	-	+	-	+	+	-	+	+	-	-	+	+	-	-	+	-	+	
JSR-22H	+	-	-	-	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	+	+	+	-	
XTR-22	+	-	+	-	+	+	+	-	+	-	+	+	-	-	-	-	-	-	+	+	+	+	+	-	
TSL-2	-	-	+	-	-	+	+	-	-	-	+	-	+	-	-	-	-	-	+	-	+	+	-	+	
JSR-17S	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	
ATR-13	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	
WIL-6	+	-	-	-	+	+	+	+	+	-	+	+	-	+	+	-	-	+	+	+	+	+	+	-	
NSL-5	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	-	+	-	-	-	
NSL-9	-	-	-	-	+	-	-	+	-	+	-	+	+	+	+	+	+	+	-	-	+	+	+	-	
NSL-15	+	-	+	-	+	+	-	+	+	-	-	+	+	+	+	+	-	+	+	+	-	+	+	+	
NSL-16	+	-	-	+	+	+	-	+	-	-	-	-	+	-	-	-	-	+	+	+	-	+	+	+	
REW-5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	
REW-7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
REW-10	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	
REW-11	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	+	+	-	+	
JWR-26C	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	+	+	-	+	
% Discordancy* 28 hybrids		38	31	38	7†	35	37	30	32	38	46	32	54	46	43	48	50	57	26	50	50	44	36	32	

DNA was isolated²² from an aliquot of cells at the same passage that karyotypic and enzyme analyses were performed. The presence of G8 sequences was determined by hybridization of the probe to filters containing *Hind*III-digested (first seven hybrids) or *Eco*RI-digested (remaining hybrids) cell hybrid DNA. Under the hybridization conditions used²² G8 did not detect hybridizing fragments in mouse DNA. A hybrid cell was considered positive for a given human chromosome only if it was present in greater than 10% of the cells. Some hybrids were made from fibroblasts containing the following translocation chromosomes: ATR-5/X, (5pter → 5q35:Xq22 → Xqter); JSR-7q⁻, (7pter → 7q22); NSL-17/9, (17qter → 17p11::9q12 → 9qter); 9/19, (9pter → 9q12::17p11 → 17pter); JSR-2/1, (2pter → 2q37::1p21 → 1pter); 1p⁻, (1qter → 1p21::2q37 → 2pter); TSL-3/17, (3qter → 3p21::17p13 → 17pter); XTR-X/3, (Xpter → Xq28::3q21 → 3qter).

* Twenty-eight additional hybrid cells were analysed for their chromosome content using only marker enzymes previously assigned to each human chromosome. The references for the gene assignments and assay methods are given in ref. 18. The markers tested are: chromosome 1, adenylate kinase-2, peptidase-C; 2, malate dehydrogenase (soluble), isocitrate dehydrogenase (soluble); 3, aminoacylase-1, DNA segment (D3S1); 4, peptidase S; 5, hexosaminidase B; 6, malic enzyme, superoxide dismutase (mitochondrial); 7, phosphoserine phosphatase, beta-glucuronidase; 8, glutathione reductase; 9, adenylate kinase-1, aconitase (soluble); 10, glutamate-oxaloacetate transaminase; 11, esterase-A4, lactate dehydrogenase-A; 12, lactate dehydrogenase-B, peptidase-B; 13, esterase-D; 14, nucleoside phosphorylase; 15, mannose phosphate isomerase, pyruvate kinase (muscle form); 16, adenine phosphoribosyl transferase; 17, galactokinase; 18, peptidase-A; 19, glucose phosphate isomerase, peptidase-D; 20, adenosine deaminase; 21, superoxide dismutase (soluble); 22, aconitase (mitochondria), DNA segment (D22S1); X, glucose-6-phosphate dehydrogenase, phosphoglycerate kinase.

† The two discordant clones were positive for G8 and negative for peptidase-S. These discordancies are probably due to the greater sensitivity of Southern blot hybridization compared to the peptidase-S stain.

Table 2 lod scores

	Recombination fraction (θ)					
	0.0	0.05	0.1	0.2	0.3	0.4
Huntington's disease against G8	A 1.81	1.59	1.36	0.90	0.48	0.16
	V 6.72	5.96	5.16	3.46	1.71	0.33
	T 8.53	7.55	6.52	4.36	2.19	0.49
Huntington's disease against MNS	-∞	-3.22	-1.70	-0.43	-0.01	0.07
Huntington's disease against GC	-∞	-2.27	-1.20	-0.32	0.00	0.07
G8 against MNS	-∞	-8.38	-3.97	-0.55	0.45	0.37
G8 against GC	-∞	-2.73	-1.17	-0.08	0.14	0.08

A, American pedigree; V, Venezuelan pedigree; T, total.

A 99% confidence interval (in this case one-sided) for the distance between the Huntington's disease locus and G8 is computed as the value of θ at which the likelihood is 100 times less than its maximum value, that is 2 log units less than 8.53. The lod score at 10 centimorgans (cM) is 6.52. Thus the 99% confidence interval is 0-10 centimorgans. One can also obtain a confidence interval based on the total number of non-cross-overs. However, in these Huntington's disease pedigrees this approach is not feasible because at-risk individuals cannot be determined as crossovers.

The lod scores for both Huntington's disease and G8 against MNS (located between 4q28 and 4q31) and GC (located between 4q11 and 4q13) suggest that these latter markers are not close to either the Huntington's disease (or G8) locus. These

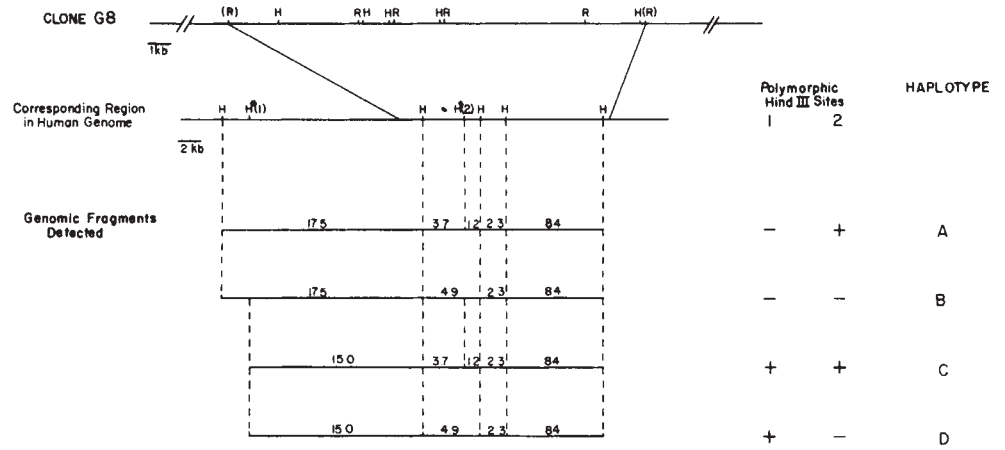
results eliminate a substantial portion of chromosome 4 as the possible region containing the Huntington's disease gene.

Implications

The discovery of a DNA marker genetically linked to the Huntington's disease locus has profound implications both for investigations of the basic gene defect and for clinical care. Certain questions must be resolved, however, in order to clarify the clinical applicability of this finding, considering the benefits and hazards involved in presymptomatic testing²⁰.

The data in this study were obtained from only two families, although they were from different ethnic backgrounds. Further study is required to determine whether Huntington's disease is

Fig. 4 Restriction map of the G8 insert and the corresponding region of chromosome 4. The restriction maps of the G8 insert and corresponding region of the genomic DNA were determined as described in the text. (H, *Hind*III sites; R, *Eco*RI sites). The polymorphic *Hind*III sites 1 and 2 are labelled H*(1) and H*(2). The two *Eco*RI sites bordering the insert are placed in parentheses as they were created during the cloning procedure¹⁵ and are not present in genomic DNA. Restriction sites in the Charon 4A vector arms are not shown. The genomic fragments generated by *Hind*III digestion that are detected by hybridization to the G8 probe are shown for each haplotype. The major allele due to polymorphism at *Hind*III site 1 is 17.5 kb fragment resulting from the absence of cleavage (-). The minor allele, as 15.0 kb fragment, results from the presence of cleavage (+) at this site. The major allele due to polymorphism at *Hind*III site 2 occurs when the site is present (+) yielding fragments of 3.7 and 1.2 kb. The minor allele in this case is a 4.9 fragment occurring when site 2 is absent (-). The haplotypes were named as follows: A, major allele at site 1, major allele at site 2; B, major allele at site 1, minor allele at site 2; C, minor allele at site 1, major allele at site 2; and D, minor allele at site 1, minor allele at site 2. It should be noted that the gel pattern for the combination AD is identical to that for BC. These can only be distinguished if the linkage phase of the alleles at sites 1 and 2 is determined by typing immediate relatives of the individual in question.



genetically heterogeneous (that is, produced by a variety of different mutations at different loci). Heterogeneity is unlikely given the exceedingly low mutation rate for the illness and clear migration patterns of the Huntington's disease gene from Europe to other parts of the World, but this possibility must certainly be tested¹.

It is equally important to assess the value of this marker for diagnosis and future research by determining how closely linked it is to the Huntington's disease locus. No obligate crossover between G8 and Huntington's disease has yet been found, and an estimate of the 99% confidence interval for the genetic distance separating the two loci extends to 10 cM. Refining the accuracy of this estimate requires testing many more individuals. Even if the G8 marker ultimately proves to be of limited utility for diagnosis, it will now be possible to use chromosome-specific cloning techniques to generate many more markers in the same region and to determine their linkage relationships to the Huntington's disease locus^{7,21}. The development of many markers closely linked on chromosome 4 will increase the potential for presymptomatic testing.

If genetic heterogeneity can be excluded, and G8 is sufficiently close to the Huntington's disease locus, it can be used in a linkage analysis to provide presymptomatic and prenatal determination of Huntington's disease gene carriers in that fraction of families that are appropriately informative. Sufficient family members must be available for analysis and must be segregating the G8 marker for the test to be successful. We are presently attempting to maximize the degree of heterozygosity, and therefore the informativeness of this locus, by searching for additional RFLPs with the G8 probe.

If the G8 marker maps quite close to the Huntington's disease gene then linkage disequilibrium may exist between the two loci. If this were the case, then it is possible the G8 marker could lead to a screening test for Huntington's disease carriers within known Huntington's disease pedigrees rather than a linkage test. It should be noted, however, that in the two families

tested here, one American and one Venezuelan, the Huntington's disease gene segregates with a different G8 haplotype in each case (A and C respectively).

Clearly caution must be exercised in considering clinical applications of the G8 marker, but its application to Huntington's disease research is immediate. The discovery of a marker linked to the Huntington's disease gene makes it feasible to attempt the cloning and characterization of the abnormal gene on the basis of its map location. Understanding the nature of the genetic defect may ultimately lead to the development of improved treatments. Furthermore, this study demonstrates the power of using linkage to DNA polymorphisms to approach genetic diseases for which other avenues of investigation have proved unsuccessful. It is likely that Huntington's disease is only the first of many hereditary autosomal diseases for which a DNA marker will provide the initial indication of chromosomal location of the gene defect.

We thank Dr David Housman (MIT) for invaluable assistance during the initiation of this project, Wendy Hobbs and Roya Rashtchian for preparation of DNA and help with G8 typing, Dr T. Maniatis (Harvard) for supplying his human genome library, Karen Rouso for help in preparation of the manuscript, and Roger Eddy, Mary Jane Barnart, Sandy Wiktorski and Leslie Daugherty for technical assistance. We also thank the members of the Venezuela Collaborative Huntington's Disease Project. A nucleus of this group deserves special recognition for their perseverance and dedication without which the pedigree and tissue collection could not have been accomplished: Fidela Gomez, Iris de Quiroz, Simon Starosta, Helen Travers, R. Stephen Uzell, Richard Erbe, Maria Villa Lobos and Humberto Moreno. This work was supported by NS16367 (Huntington's Disease Center Without Walls) NINCDS grant and NIH grant GM26659, and grants provided by the Hereditary Disease Foundation, the Julieanne Dorn Fund for Neurological Research, the Vollmer Foundation, the Regenstein Foundation and the March of Dimes grant 1-692.

Received 5 October; accepted 20 October 1983.

- Hayden, M. R. *Huntington's Chorea* (Springer, New York, 1981).
- Wexler, N. S. *Genetic Counseling: Psychological Dimensions* (ed. Kessler, S.) 190-220 (Academic, New York, 1979).
- Bruyn, G. W. *Prog. Brain Res.* **55**, 445-464 (1982).
- Martin, J. B. *Nature* **299**, 205-206 (1982).
- Pericak-Vance, M. A. *et al. Adv. Neurol.* **23**, 59-72 (1979).
- Volkers, W. S. *et al. Ann. hum. Genet.* **44**, 75-79 (1980).
- Housman, D. & Gusella, J. F. *Molecular Genetic Neuroscience* (eds Schmitt, F. O., Bird, S. J. & Bloom, F. E.) 415-424 (Raven, New York, 1982).
- Botstein, D., White, R. L., Skolnick, M. & Davis, R. *Am. J. hum. Gen.* **32**, 314-331 (1980).
- Kan, Y. W. & Dozy, A. M. *Lancet* **ii**, 910 (1978).
- Solomon, E. & Bodmer, W. F. *Lancet* **i**, 923 (1979).
- Murray, J. M. *et al. Nature* **300**, 69-71 (1982).
- Wexler, N. S. *et al. Cytogenet. Cell Genet.* (in the press).

- Shoulson, I. & Fahn, S. *Neurology* **29**, 1-3 (1979).
- Gusella, J. *et al. Cytogenet. Cell Genet.* (in the press).
- Lawn, R. M., Fritsch, E. F., Parker, R. C., Blake, G. & Maniatis, T. *Cell* **15**, 1157-1174 (1978).
- Gusella, J. F., Tanzi, R., Anderson, M. A., Ottina, K. & Watkins, P. in *Banbury Report 14: Recombinant DNA Applications to Human Disease* (eds Caskey, C. T. & White, R. L.) 261-266 (Cold Spring Harbor Laboratory, New York, 1983).
- Ott, J. *Am. J. hum. Genet.* **26**, 588-597 (1974).
- Shows, T. B., Sakaguchi, A. Y. & Naylor, S. L. *Adv. hum. Genet.* **12**, 341-452 (1982).
- Naylor, S. L., Gusella, J. & Sakaguchi, A. Y. *Cytogenet. Cell Genet.* (in the press).
- Wexler, N. S. in *Progress in Medical Genetics*, Vol. 7 (ed. Barton Childs) (Saunders, Philadelphia, in the press).
- Gusella, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 2829-2833 (1980).
- Naylor, S. L. *et al. J. molec. Biol.* **98**, 503-517 (1983).
- Gusella, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 5239-5243 (1979).
- Varsanyi-Breiner, A. *et al. Gene* **7**, 317-334 (1979).