regulatory factor dictating the observed pattern of the processing of the calcitonin-CGRP transcript.

Identification of the factor (or factors) which determines the tissue-specific pattern of splicing and poly(A) site selection should allow for its isolation and detailed characterization, a prerequisite for understanding the mechanisms of its actions. On the basis of the potential utilization of this type of control in certain developmental loci of *Drosophila*, it is possible that alternative RNA processing is, in fact, a critical mechanism for control of neuronal development. While one consequence of such developmental regulation is that a single neuroendocrine gene can generate distinct, tissue-specific mRNA's, each encoding a different neuropeptide, there could be additional, widespread consequences with regard to productive or nonproductive processing of the transcripts of a large number of active brain transcription units. In such a case, one might envision that, in certain transcription units, selection of alternative poly(A) sites might result in (or from) a splicing pattern generating an unstable or an unproductive transcript. Only by evaluating the expression of genes encoding the factor (or factors) responsible for alternative RNA processing events in the brain, such as that required in the case of calcitonin-CGRP gene expression, can these questions be systematically addressed.

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50. We thank our colleagues C. Lin, L. Stolarsky, V. Jonas, J.-J. Mermod, E. S. Ong, L. Swanson, P. Sawchenko, W. Vale, M. Brown, and J. Rivier for their contributions to these investigations.

DNA Markers for Nervous System Diseases

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One of the major challenges in modern biology is to unravel the mystery of how the human brain functions. A method that has proved fruitful in other systems is analyzing mutations in order to define the normal mechanisms they disrupt. In man, genetic alterations affecting brain function are commonly expressed as inherited neurological or behavioral diseases. The development of recombinant DNA techniques has advanced our understanding of many human genetic diseases by permitting the isolation and sequencing of specific defective genes. It is now relatively straightforward to clone and characterize any gene whose product is known. Unfortunately, in most genetic diseases, the primary protein defect has yet to be identified. The task of finding a specific protein defect among the 40,000 to 200,000 possible proteins encoded in the human genome is enormous unless additional information is available to aid in the search. We have therefore embarked on the development of an alternative approach to the investigation of human genetic diseases. We intend to base our strategy for isolating a defective gene on its location in the human genome. The identification of an altered protein by analysis of the gene

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that encodes it would be the reverse of the current approach of cloning the disease gene only after first defining the primary protein defect.

Huntington's Disease

The first step in applying our proposed strategy to a genetic disease is to determine the chromosomal location of the mutant gene. We chose Huntington's disease (HD), an autosomal dominant neurodegenerative disorder, as a model system for developing this approach (*1, 2*). Huntington's disease is characterized by apparently normal central nervous system development followed by premature selective neuronal cell death, most notably in the basal ganglia. While chorea is the most widely recognized symptom of this disorder, intellectual and psychological manifestations are also present. These can include memory loss, mood shifts, personality changes, and chronic depression. Although the mutant gene is completely penetrant, onset of symptoms does not usually occur until the third to fourth decade of life, often after the defect has already been passed on to children. The disease progresses for 15 to 20 years, until the HD victim is totally disabled physically and is unable to communicate. Death commonly results from heart disease due to the incessant movements of the patient or from pneumonia secondary to aspiration.

The prevalence of HD is approximately 10 per 100,000 in most Caucasian populations, but it has a devastating impact on a far greater number of individuals, including the children "at risk" and family and friends of patients. In the 112 years since George Huntington first described HD (*3*), investigations have failed to identify the primary biochemical defect in this mystifying disease. There is currently no effective therapy to combat the inexorable deterioration of its victims.

Genetic Linkage and DNA Markers

In order to define the chromosomal map position of the HD gene, we combined the traditional technique of genetic linkage analysis with the power of modern recombinant DNA techniques (*4, 5*). This strategy, which is outlined below, could also be applied to a large number of other inherited neurological disorders and is not limited by any assumptions concerning the nature of the primary defect (*6, 7*). We are currently using this method to investigate familial Alzheimer's disease (*8*), peripheral and central neurofibromatosis (*9, 10*), von Hippel-Lindau disease (*11*) and autosomal dominant dystonia musculorum deformans (*12*).

When a gene is present in an individual in two allelic forms that are readily distinguishable (for example, blood group alleles A and B), each allele can be used to trace the inheritance of the particular chromosome that carries it (Fig. 1). When two genes are close to each other on the same chromosome, they will frequently be passed on to progeny together. Occasionally, a recombination event between the two genes will reassort the linked alleles. The frequency with which such recombination events occur between two genes can be used as a measure of the distance that separates their loci.

The HD victim has two different alleles at the HD locus since one chromosome carries the normal and the other the defective allele. We performed genetic linkage studies to find another genetic characteristic that was very frequently inherited with HD. These investigations involved tracing the inheritance of genetic markers that show frequent allelic variation (polymorphism) in large HD families and correlating the data with the inheritance of the disease gene. Such studies, in which standard protein polymorphisms were used had been performed in the past without success, although they had eliminated the location of the HD gene from 15 percent of the genome (*13, 14*). Since the disease locus clearly did not reside in the X chromosome (which constitutes about 6 percent of the human genome), we were faced with searching for the HD gene in the remaining 80 percent of the genome. Unfortunately no additional proteins displaying frequent polymorphism had been described. We therefore turned to recombinant DNA methods to generate new polymorphic genetic markers that could be used in genetic linkage studies.

The basic tool of recombinant DNA technology, the restriction endonuclease, is capable of making a double-stranded cut in a DNA molecule wherever its particular recognition sequence occurs. An alteration in the human genome as small as a single base change, located in the DNA at one of these recognition sequences, will eliminate cleavage at that site. Restriction endonucleases can therefore be used as a method of monitoring heritable sequence variation in human DNA. In practice, these DNA polymorphisms are detected as differences in the sizes of restriction fragments (restriction-fragment-length polymorphisms or RFLP's) (*5*) generated at a particular locus by digestion with a given enzyme, as shown in Fig. 2. Human DNA is digested with a restriction enzyme, and the resulting fragments (approximately 1,000,000 for a typical enzyme) are fractionated according to size by agarose gel electrophoresis. DNA from the gel is transferred to a solid filter support (Southern blotting) (*15*) and hybridized with a cloned radioactive sequence of human DNA from one particular position in the genome. The radioactive probe anneals to its complementary sequence in the genomic digest and is therefore retained on the filter only at positions marking the fragment sizes generated from that locus. These positions are detected by autoradiography of the washed filter.

The DNA sequence variations detected with this procedure display Mendelian inheritance and can be used as genetic markers in linkage studies. When our attempts to map the HD locus began in 1980, there were but three DNA markers available. In the past 4 years, this number has increased to more than 150, far more than the 25 to 30 useful protein markers (*16*). DNA markers also have a number of advantages over traditional protein polymorphisms. First, a DNA sequence need not be expressed as a protein to display useful polymorphism. DNA polymorphisms can occur in any type of sequence (for example, intervening sequences or flanking sequences) and make good genetic markers as long as they can be detected at only one chromosomal location. In fact, most DNA markers now available have been generated from anonymous DNA sequences whose function has not been explored. Second, DNA markers can potentially be found in all regions of the genome, wherever a single-copy sequence of DNA exists.

Summary. Recombinant DNA technology has provided a vast new source of DNA markers displaying heritable sequence variation in humans. These markers can be used in family studies to identify the chromosomal location of defective genes causing nervous system disorders. The discovery of a DNA marker linked to Huntington's disease has opened new avenues of research into this disorder and may ultimately permit cloning and characterization of the defective gene.

DNA markers have now been described for all human chromosomes. Third, the testing process for DNA markers is iterative, allowing reuse of the same filter and therefore the same DNA sample with multiple probes. Finally, the application of additional restriction enzymes or cloning of genomic DNA adjacent to the locus to generate additional probes, or both of these techniques, can result in the detection of multiple variant sites, thereby generating highly informative multiallele marker systems.

Family Studies

It has been proposed that DNA markers could be used to construct a complete linkage map covering all regions of the human genome (*5*). The rate of discovery of new RFLP's makes it likely that such a map will be available in the next few years. The existence of a human linkage map will not be sufficient, however, to enable these DNA markers to locate a genetic disease gene. The investigator must first identify very large families in which the disease gene is segregating in order to obtain a statistically significant score of coinheritance of the marker and the disease.

In our search for the HD locus, we used two pedigrees. The first was an American family obtained through the National HD Research Roster at Indiana University (*17*). The second was a very large family whose members live in three communities along the shore of Lake Maracaibo in Venezuela. This family was made available to us by the United States–Venezuela Collaborative HD Project (*18*). We expected to test several hundred DNA markers before finding one linked to the HD gene, and we therefore required a permanent source of DNA from family members. Lymphoblastoid cell lines were therefore established from 27 members of the American family and over 550 members of the Venezuelan family.

With the availability of large HD families and of ever-increasing numbers of DNA markers, the eventual discovery of the map location of the HD gene became a virtual certainty. Fortunately, soon after beginning our screen for a marker linked to the HD locus, we discovered that G8, one of the new RFLP markers we had generated, showed close linkage to the HD gene in both of our families (*18*).

The G8 Marker

The G8 probe used in our initial studies consisted of 17.6 kilobases (kb) of single-copy human DNA cloned in a phage vector. It detected two different RFLP's when hybridized to a Hind III digest of genomic DNA. The polymorphisms resulted from two variable Hind III sites, each of which is absent on some chromosomes presumably because of a sequence difference that affects the specific AAGCTT sequence (A, adenine; G, guanine; C, cytosine; T, thymine) at the potential site of cleavage. In our more recent investigations, we have used a plasmid subclone of G8 (pK082), containing a 5.5-kb Eco RI fragment from the left edge of the G8 insert. The subclone probe detects the same two Hind III polymorphisms (Fig. 3) without the interference of any of the invariant fragments hybridizing to the original phage clone. A fragment of either 15 or 17.5 kb is seen, depending on the presence or absence of polymorphic site 1. Presence or absence of polymorphic site 2 result in fragments of 3.7 and 4.9 kb, respectively. There are four possible patterns of fragments generated from these two polymorphic sites. These four patterns have been termed haplotypes A, B, C, and D (Fig. 3). Each haplotype has a significant frequency in the general population, making G8 a highly informative four-allele locus. A screen of genomic DNA's from 51 unrelated individuals indicates that the A haplotype is present on approximately 50 percent of chromosomes in the general population. The frequencies of the B, C, and D haplotypes were 16, 25, and 9 percent, respectively. In all, 35 of the 51 persons tested were heterozygous for this DNA marker. The actual gel patterns seen for individuals representing each of the ten possible genotypes generated from these four haplotypes is shown in Fig. 4.

Our first report of genetic linkage of the G8 marker to HD was based on typing results obtained with the American family and a small portion of the Venezuelan pedigree (*18*). In that study, no obligate recombinants were detected between the marker and the disease gene, indicating 100 percent coinheritance. In the American family, the HD gene was on a chromosome carrying the A haplotype of the G8 marker, whereas in the Venezuelan pedigree it traveled with the C haplotype. In our subsequent typing of additional members of the Venezuelan pedigree, we detected a single recombination event (Fig. 5) that transferred the HD gene to a chromosome containing the A haplotype at the G8 locus. The best estimate for the distance separating the two loci, based on data from these two families, currently stands at 2 centimorgans (cM) (2 percent recombination or approximately 2000 kb). This figure is very imprecise since it is based on a single recombination event, and the estimate may well increase as data is accumulated from more families and additional recombinants are found.

The use of a panel of human-mouse hybrid cells in which each clone has a different complement of human chromosomes originally allowed the assignment

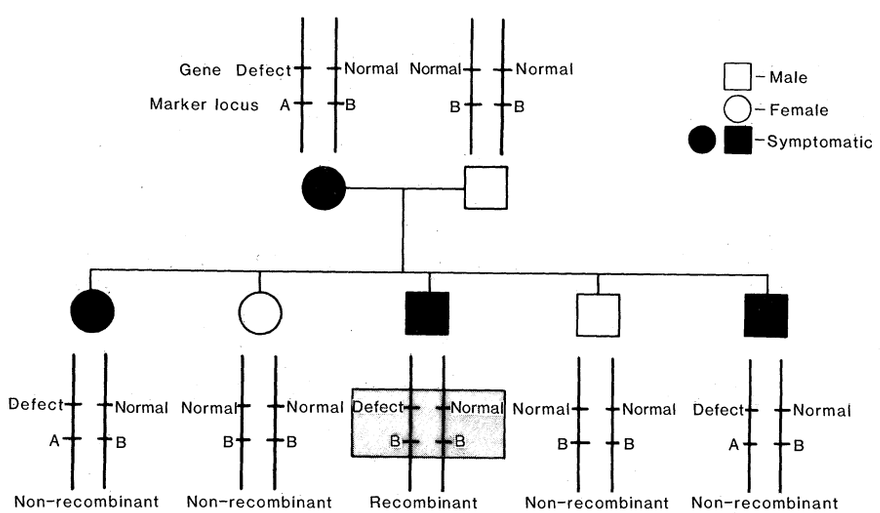


Fig. 1. Genetic linkage and recombination. A polymorphic marker locus with two allelic forms, A and B, maps close to a defective locus. In this small family, the mother is heterozygous at both the marker locus and the disease locus, whereas the father is homozygous at both sites. The chromosome carrying the defective gene resides on a chromosome with an A allele at the marker locus. Since the marker and disease loci are genetically linked, most children who inherit the disease gene will receive the A allele from the mother. When a recombination event occurs in the mother between the disease locus and the marker locus, the defective gene is transferred to a chromosome bearing the B allele of the marker.

of the G8 marker and, by inference, the HD gene to human chromosome 4 (*18*). Subsequent application of the same method with hybrid cells containing only portions of chromosome 4 as translocation chromosomes has resulted in the refinement of this assignment to the short arm of the chromosome (data not shown). This localization is consistent with the absence of genetic linkage that we have observed in our family studies between these two loci and the traditional markers MNS and GC, both located on the long arm. More precise mapping of the G8 locus awaits the application of in situ hybridization techniques and the use of chromosomes from patients with Wolf-Hirschorn syndrome, a congenital anomaly resulting from partial deletion of the short arm of chromosome 4 (*19*).

Consequences of Mapping the HD Gene

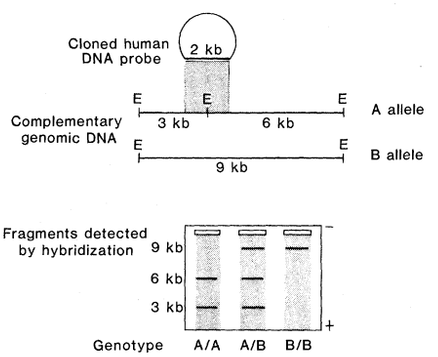
The discovery of a DNA marker linked to HD will provide the means to answer a number of questions concerning the genetics and expression of this devastating disease. First, the typing of additional HD families will show whether the disease gene is located on chromosome 4 in all cases or, conversely, whether mutation at another locus can duplicate the HD phenotype. The existence of more than one locus causing similar symptoms is known for some other human diseases, and nonallelic heterogeneity in HD might profoundly alter the views of both clinicians and investigators toward this seemingly homogeneous disorder.

A second feature of HD that can be explored is the frequency of new mutations; HD is unusual among the more common dominant genetic diseases in that it apparently displays an exceedingly low mutation rate (*20, 21*). In fact, there is not a single unequivocal case reported of a new mutation causing HD. In the cases described in which there is no family history of the disease, nonpaternity is often a possible explanation. In reports in which paternity has been established, there is as yet no proof that the lesion is genetic. This would require finding a "sporadic" HD case in which at least one child of the affected individual with no family history of the disease also shows symptoms of the disorder. The fact that no new mutation to HD has ever been described may be a reflection of the difficulty of proving such a mutation, but it also suggests the possibility that the HD alleles in most or all of the HD families living today may share a common origin. The frequency of HD is highest in Caucasian populations, and the spread of HD around the world coincides with the historical patterns of travel and colonization from areas in Western Europe (for example, England and Holland) where the disease is common today. The relative infrequency of the disorder in Orientals and its effective absence in African blacks of pure ancestry also support the idea of a single original mutation event generating the HD gene (*1*).

The discovery of the chromosomal map position of the HD locus should permit testing of this hypothesis by use of the phenomenon of linkage disequilibrium. When a new disease-producing mutation occurs, the specific chromosome on which the event occurs has one particular complement of alleles at polymorphic sites in the immediate vicinity of the mutation. For a number of generations thereafter, the probability of finding this set of alleles on a chromosome containing the disease gene is much higher than would be expected from the frequency of the alleles in the population. The disease gene and alleles in the vicinity are said to be in "linkage disequilibrium." As many generations pass, recombination events between these other sites and the mutation—and the occurrence of new disease-producing mutations at the same locus on other chrom somes—reduce the significance of t original association. The frequency particular alleles at these other sites chromosomes containing the mutati approaches the frequency seen for thc alleles in the general population. T markers and the disease locus are th said to be in "equilibrium."

If most of the HD families share common mutation, polymorphic marke close to the HD gene are likely to displ some degree of linkage disequilibriu with the HD locus, with a higher th expected frequency for alleles that we present on the original chromosor where the mutation occurred. The c gree of disequilibrium observed will c pend in part on how long ago the H mutation occurred and on how far t marker locus is from the disease gene

The third issue that can now be inve tigated with the use of markers linked HD is the "dominant" transmission this disease. There is as yet no case o human disease that shows a complete dominant pattern of inheritance with significant difference in phenotype b tween individuals who carry one or tv copies of the mutant gene (*22*). In in viduals who have been homozygous a dominant disease locus, the result h either been symptoms far worse th those in the heterozygous state, or



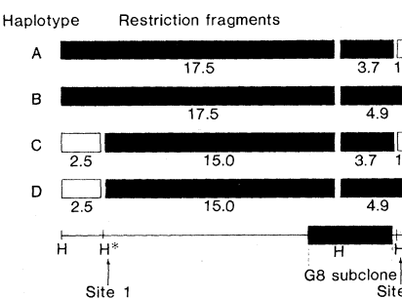


Fig. 2 (left). Restriction-fragment-length po morphism. In this example of an RFLP cloned 2-kb single-copy sequence of human DNA is capable of detecting the presence absence of a polymorphic Eco RI (E) site. The presence of the variable site results in Eco fragments of 3 and 6 kb. This is the A allele at this locus. The B allele results when the cent Eco RI site is missing because of the absence of the exact recognition sequence GAATTC. T site could have been lost as a result of a single base change in the genomic DNA. The B allele recognized by a 9-kb Eco RI fragment. Individuals can be typed at the marker locus by digesti their genomic DNA to completion with Eco RI, resolving the DNA fragments by agarose electrophoresis, transferring the DNA from the gel to a solid filter support, and hybridizing w the radioactive probe (*15*). The position at which the probe hybridizes to a complementa sequence is then detected by autoradiography. The inset shows the pattern observed for each the three possible genotypes at the marker locus. If the probe molecule did not cross t variable Eco RI site but was derived totally from the region of the 6-kb fragment, then the A a B alleles would be the 6-kb fragment and 9-kb fragment, respectively. The 3-kb fragment wou therefore not be detected by the probe in this situation. Fig. 3 (right). Haplotypes at the locus. The G8 marker results from the detection of two polymorphic Hind III sites, each givi two possible alleles (presence of the site or absence of the site). The combination of alleles fr each of these tightly linked sites is referred to as a haplotype. At the G8 locus, there are fc possible haplotypes, A, B, C, and D. The subclone of G8 used as probe (pK082) is depicted a the fragments it detects for each haplotype are shaded. The unshaded fragments are al characteristic of each haplotype but are not overlapping with the probe, and therefore are r seen on hybridization.

thality. It would be of considerable interest to determine whether the HD mutation might display a completely dominant phenotype. One of the original motivations for investigating the large Venezuelan HD focus was the possibility of discovering an HD homozygote. It should now be possible to determine, by typing members of selected sibships in which both parents have HD, whether an individual homozygous for the HD gene exists and whether the symptoms or age of onset of the disease are altered in such a double-dose case.

Finally, the generation of additional DNA markers linked to the HD gene will also permit a test to be developed to determine whether asymptomatic individuals at risk for developing the disease are in fact gene carriers. Each child of an HD parent is at 50 percent risk for having inherited the HD gene. Most gene carriers will remain asymptomatic, however, until middle age. By tracing the inheritance of these DNA markers it will be possible to decide which children have not inherited the HD gene and are not "at risk," and which children are actually presymptomatic HD gene carriers. The same linkage test could be used for the prenatal detection of HD status. Although the use of such a test as a clinical service must be delayed until the question of nonallelic heterogeneity has been decided, the procedure could be used to explore some of the effects of the HD gene in individuals who are not symptomatic on neurologic examination. The discovery of a genetic marker has therefore made possible a more accurate assessment of the biochemical, psychological, and intellectual differences that may be seen in at-risk individuals.

A major difficulty in the past has been that any difference purported to be characteristic of HD in presymptomatic individuals could be investigated only by determining whether a 50:50 ratio was obtained for the phenotype in a pool of at-risk subjects. The significance of such results was unclear since which individuals actually carried the HD gene could not be shown. Linked DNA markers will now make it possible to decide—long before the first manifestation of symptoms—which at-risk individuals do carry the HD allele. Potential areas to be explored with the linkage test include memory deficit, psychological and intellectual parameters (*23, 24*), osmotic fragility of erythrocytes (*25*), and other biochemical phenotypes expressed in peripheral tissues, such as radiation sensitivity (*26*) and glutamate toxicity. Perhaps most interesting will be the use of positron emission tomography (*27*) to determine at what point before the onset of symptoms decreased cellular activity in the basal ganglia can be measured.

The importance of pursuing these studies is twofold. First, they may lead to the characterization of physical, psychological, or intellectual tests that can be performed in conjunction with the marker test to improve the accuracy of presymptomatic diagnosis, and ultimately, to provide a prognosis with respect to age of onset and rate of progression of the disorder. Second, the biochemical and physical determinations may yield valuable information on the timing of expression of the HD gene and, potentially, a peripheral cell type expressing the primary defect. Both of these would improve the likelihood of successfully identifying the HD gene.

Obtaining DNA markers linked to other genetic disease loci will allow similar biochemical and behavioral investigations to be performed for these disorders. The availability of improved diagnosis, especially prenatal diagnosis, is another likely result of the successful mapping of a genetic lesion. Perhaps the most important consequence of such discoveries, however, is that they allow the investigator to design cloning strategies for isolating the disease gene by knowing its chromosomal map location. The cloning of the HD gene with a strategy based on its location on chromosome 4 could act as a model for the development of techniques that could then be applied to other genetic diseases.

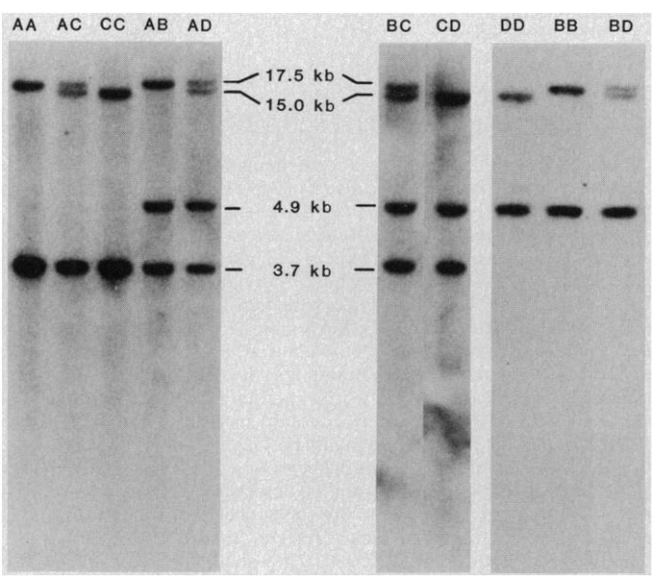


Fig. 4. Autoradiograph showing each of the ten possible genotypes at the G8 locus. The pattern of fragments observed for each combination of haplotypes can be predicted from Fig. 3. The pattern observed for the genotype AD is identical to that for BC; they can be distinguished only by typing close relatives of the individual with the ambiguous genotype. Digestion of DNA, agarose gel electrophoresis, DNA transfer, hybridization, and autoradiography were performed as described (*17*), with the 5.5-kb Eco RI fragment (subcloned into the plasmid vector pBR328) from the G8 phage insert (see Fig. 3).

Steps Toward Cloning the HD Gene

Typing of additional pedigrees with the G8 marker will determine whether another mutant locus exists in some families but will also serve another purpose. In families in which the HD gene is linked to the marker, occasional recombination events between the two loci will be revealed. Each of these recombination events marks a point closer to the HD gene than the G8 locus is. A similar procedure could be followed for a polymorphic marker on the other side of the HD gene. The isolation of such a flanking marker, by cloning of anonymous DNA fragments from the short arm of chromosome 4, is the next step in our attempt to clone the HD gene. Each probe that detects an RFLP will have to be tested for segregation in HD families already typed with G8 to determine whether it is a flanking marker or is on the same side of the disease locus as G8. Once a useful flanking marker is found, it will be possible to use it in conjunction with G8 to identify recombination events on both sides of the HD gene. The nearest such events on either side of the disease locus should genetically bracket it to the minimum region that must be searched to identify the primary defect. The size of this region will depend on the number of informative meiotic events that can be analyzed. If 200 events are typed, the bracketed region should be approximately 1 cM or 1000 kb in size.

Subsequent steps in finding the HD

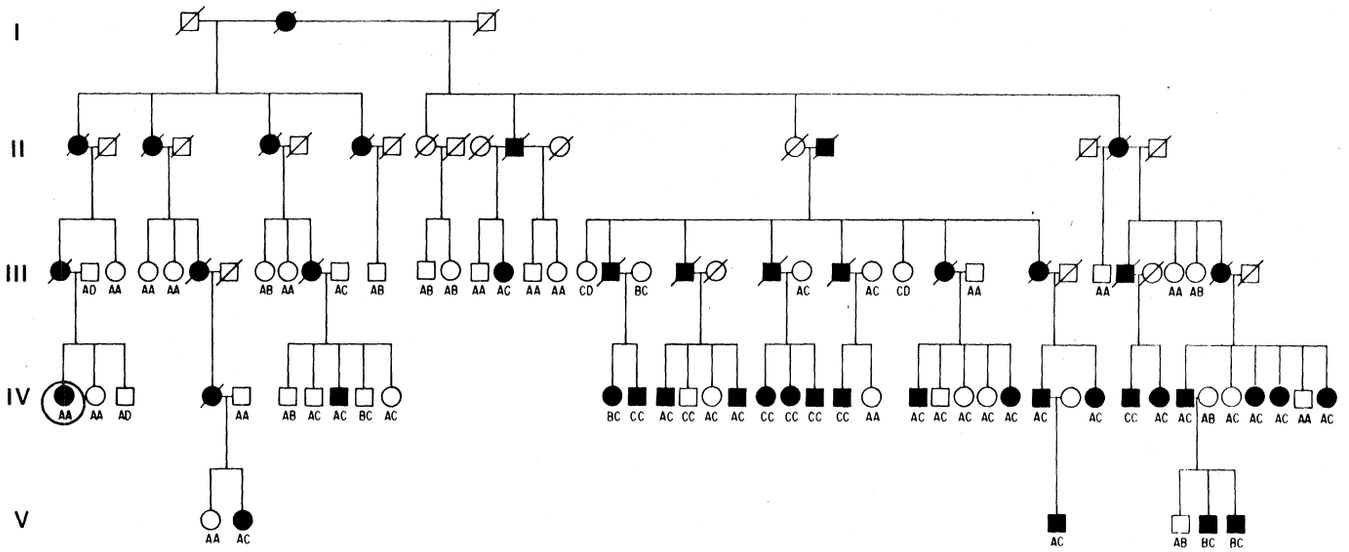


Fig. 5. A portion of the Venezuelan HD pedigree. The legend for this figure is that shown in Fig. 1 (deceased individuals have a diagonal slash through the symbol). This subset of members of the Venezuelan family illustrates the inheritance of the HD gene on a chromosome bearing the C haplotype at the G8 locus. Typing of these individuals was performed as shown in Figs. 3 and 4. A single individual representing a recombination event (shown in the shaded circle) was found in this portion of the pedigree. Lod score analysis for the entire Venezuelan family (not shown here) combined with that for the American family (*17*) now yields a maximum score of 12.1 corresponding to odds of greater than 1 trillion to 1 in favor of linkage. The best estimate of the distance from the G8 marker to the HD locus is currently 2 cM (2 percent recombination).

gene will require the cloning and analysis of all DNA in the bracketed region. When we began the HD linkage project in 1980, it was clear that fragments of DNA from specific human chromosomes could be cloned from flow-sorted human chromosomes (*28*) or from appropriate human-rodent hybrid cells (*29*). It was also well established that it was possible to "walk" along a chromosome from one point to another by isolating overlapping genomic DNA fragments from recombinant DNA libraries. Individual steps took so long, however, that a walk of 100 kb was considered an extraordinary effort. Neither of these methods, either alone or in combination, would be adequate to perform the task of isolating 1000 to 2000 kb of contiguous genomic DNA. Fortunately, in the past 4 years, several crucial technological advances have been made that now make it feasible to bridge the gap between a DNA linkage marker located a few million base pairs away and the disease locus.

One particularly helpful innovation is the introduction by Schwartz and Cantor (*30*) of pulse-field gradient electrophoresis. This technique, which is based on the application of alternating, perpendicular inhomogenous electric fields to a standard horizontal agarose gel, is capable of resolving DNA fragments of up to 5000 kb. It has already been applied to the separation of intact DNA strands from yeast chromosomes. This method could potentially be used preparatively to purify very large restriction fragments (200 to 5000 kb) produced with enzymes that cut infrequently in human DNA. These large fragments could then be digested with a second enzyme and the smaller segments cloned in an appropriate vector. The resulting library would have a very low complexity and consist solely of sequences contiguous in the human genome.

Advances in somatic cell genetic techniques might also facilitate the cloning of a stretch of DNA surrounding the disease gene. Bacterial genes, placed downstream from mammalian promoters, can provide dominant selective systems for use with mammalian cells (*31–33*). These genes can be introduced into mammalian cells in a number of different ways, including fusion with bacterial protoplasts or infection with retroviral vector. Once introduced, the selectable marker apparently integrates randomly into the chromosomes of the cell. If a cell line could be constructed with one of these dominant selectable markers integrated into the short arm of human chromosome 4 near the HD locus, this region could be stably transferred into a rodent cell. This method has already been used successfully to transfer the murine histocompatibility complex, H-2, into a hamster cell (*34*). The advantage of having a small region of human chromosome in a rodent cell is that all human sequences could then be recovered by constructing a genomic library and screening the recombinants for hybridization to human-specific repetitive sequence probes (*29*). No time-consuming chromosome walking steps would be required with this method, although additional procedures would have to be used to determine the linear order of the cloned sequences on the chromosome.

Once the bracketed region containing the disease gene has been cloned, the DNA must be analyzed to further narrow the region of search and ultimately to identify the defect. Two new techniques could be used in this task. First, denaturing gradient gels, developed by Lerman and his co-workers (*35, 36*) are capable of detecting differences in DNA sequence as small as a single base change, even when the alteration does not affect the size of a restriction fragment. The system is based on the effect that sequence differences have on the stability of a DNA molecule and the decrease in electrophoretic mobility that follows the partial melting of a double-stranded DNA molecule. These gels can be used to compare in detail the two homologous sequences or to find DNA polymorphisms in segments in which no RFLP's are detected.

Second, the availability of bacterial expression vectors that allow selective cloning of "open reading frames" (*37, 38*) should permit the isolation of all coding sequences from within the bracketed region containing the HD gene. This would not only reduce significantly the total amount of sequence to be compared to identify a protein defect, but would also provide a fusion protein expressed in the bacterial host. These fusion proteins, partly bacterially encoded and partly encoded by the human open read-

ing frames, could be used to produce antiserums to detect the corresponding human proteins and identify individual gene products. Each of these gene products would then be analyzed as the potential defective protein in HD.

The exact strategy for cloning a disease gene on the basis of its map location will no doubt differ in special details for each disorder. The methods used will probably involve some combination of these new techniques with more standard cloning and somatic cell genetic methods. With HD, new strategies may become evident as each of the techniques used is further refined and additional information is gained concerning the genetics and expression of the defective gene and its precise location on chromosome 4. The rapid pace of technological development in the field of recombinant DNA research also makes it likely that additional useful methods will become available as this work progresses. It should certainly be possible, however, to isolate the HD gene by intensive application of the techniques already at hand.

The characterization of the HD gene and its normal counterpart will certainly provide improved diagnostic capability, and may yield information that will lead to an effective therapy for this disease. Furthermore, the investigation of this specific mutation might yield significant insights into fundamental mechanisms operative in the human nervous system. Perhaps most important, however, is the likelihood that this system will pave the way for application of similar techniques to improve our understanding of many other neurogenetic disorders.

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39. Supported by grants NS16367 (Huntington's Disease Center Without Walls) and NS20012 from the National Institutes of Health, by a grant from the Hereditary Disease Foundation, and by the Julieanne Dorn Fund for Neurological Research.

Chemical Anatomy of the Brain

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The term "chemical anatomy of the brain" encompasses a large field of research. The present article includes a review of some of the histochemical-neuroanatomical methods employed in chemical neuroanatomy and some of the knowledge on systems containing classical transmitters. Among the classical transmitters discussed are the catecholamines, acetylcholine (ACh), and γ-aminobutyric acid (GABA). The more recently discovered neuropeptides and their possible role as cotransmitters or comodulators are also considered.

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Aspects of Methodology

The short history of transmitter histochemistry is to a considerable extent the history of the development of new methods. The first attempts to map transmitter-identified pathways were made by Koelle and Friedenwald (*1*) using ACh esterase (AChE) staining. Thus the enzyme responsible for the breakdown of ACh was taken as a marker for cholinergic pathways. The underlying histochemical reaction was of the metal-precipitation type, and specificity was achieved by using inhibitors of nonspecific esterases.

A different approach was taken for visualizing monoamine neurons. Here the aim was to demonstrate the transmitter itself. Based on the original work of Eränkö (*2*), studies by Hillarp, Falck, and their collaborators resulted in a reliable formaldehyde-induced fluorescence method (Falck-Hillarp technique) for demonstrating norepinephrine, dopamine, and to a lesser extent 5-hydroxytryptamine (5HT) in tissue sections (*3*). Subsequently, many modifications of the original technique were introduced (*4*). These steps improved the quality of sections and the sensitivity of the method, especially for visualizing dopamine systems.

A logical continuation of the formaldehyde fluorescence studies of catecholamine neurons was the introduction of the indirect immunofluorescence method (*5*). The work of Geffen *et al.* (*6*) as well as other studies (*7*) demonstrated that antisera to dopamine-β-hydroxylase (DBH), the enzyme converting dopamine to norepinephrine, could be used to visualize peripheral noradrenergic neurons and gland cells in the adrenal medulla. This approach was rapidly broadened to include visualization of all four enzymes in the synthesis of catecholamines: tyrosine hydroxylase, L-aromat-