

lampreys, in which the chemical component is also usually larger than the electrical component.

The reason for the increase in chemical EPSP amplitude in the regenerated synapses is not yet known. One possibility is that GI's which had been disconnected from some of their synaptic input developed denervation supersensitivity. Consistent with this is the finding that, when both GI's were below the transection, the chemical EPSP was also increased (although because of the small sample size this was not statistically verifiable), while the electrical component was not. Two other possibilities, that regenerating connections are formed closer to the cell body or include more points of synaptic contact, are both less likely because the electrical components in the regenerated synapses were reduced.

The unequivocal demonstration of functional synapse formation by regenerating neurites ideally should involve simultaneous impalement of both the pre- and postsynaptic cells because (i) it is possible that synaptic activity evoked by extracellular stimulation might result from antidromic activation of axon collaterals of cells on the same side of the lesion as the target neuron and (ii) regenerated axons might release substances diffusely into the extracellular environment and activate neurons nonsynaptically. Such direct evidence has been obtained in invertebrate ganglia (10), but in the vertebrate CNS synaptic regeneration has been suggested by less direct methods. Morphological evidence has been found for the formation of synapses by regenerating fibers in the spinal cords of lampreys (11), bony fish (12), and amphibians (13). A combination of anatomical, behavioral, and evoked-potential studies has suggested regeneration of retinotectal synaptic connections in fish and amphibians (14). Stimulation of regenerated Mauthner axons has evoked compound action potentials in ventral roots of *Xenopus* tadpoles (15). Finally, excitation of muscle nerves in the forearms of frogs that had recovered from lesions of the second dorsal root elicited short-latency EPSP's in ipsilateral motor neurons (16). The present study shows that regenerated [as opposed to collaterally sprouted (17)] axons of vertebrate CNS neurons form functional synaptic connections. This suggests that similar connections may well be formed by regenerating axons from mammalian grafts and bridges (2, 3), where the distance of axon growth is similar to that in the lamprey spinal cord.

We previously showed that the regen-

erating neurites of GI's and other neurons of the lamprey CNS grow selectively in the direction of their original paths (4, 5, 18). Whether these axons show similar specificity in their choice of postsynaptic targets remains to be determined.

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## Huntington's Disease: Two Families with Differing Clinical Features Show Linkage to the G8 Probe

**Abstract.** To test the hypothesis that interfamilial variability in Huntington's Disease (HD) is due to mutation at different loci, linkage analysis was undertaken in two large HD kindreds that differed in ethnicity, age-at-onset, and neurologic and psychiatric features. Both families showed linkage of the HD locus to the G8 probe. Several recombinants were documented in each family, and the best estimate of the recombination fraction for the two families was 6 percent with a 95 percent confidence interval of 0 to 12 percent. Although the data support the existence of a single HD locus, use of the G8 probe for presymptomatic testing in these kindreds would have resulted in a 12 percent error rate in genotype assignment at the HD locus.

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Huntington's disease (HD) (1) is an inherited neuropsychiatric disorder, characterized by gradually worsening chorea, impairment of voluntary movements, dementia, and a variety of emotional symptoms, particularly severe depression (2, 3). The symptoms progress without remission until patients are physically incapacitated and severely impaired in cognition. Death occurs 15 years after onset (on the average) usually from subdural hematoma due to head trauma or from suffocation due to aspiration of food. The disease is inherited as an autosomal dominant trait, with onset of symptoms usually delayed until middle adult life. The paternal transmission effect is well documented in HD, but its cause is not understood. Affected children of affected fathers tend to have a significantly earlier onset than the affected children of an affected mother (4).

The disease is severe and the offspring are at high risk (50 percent) for eventually having symptoms. However, because of the delayed onset, even offspring of

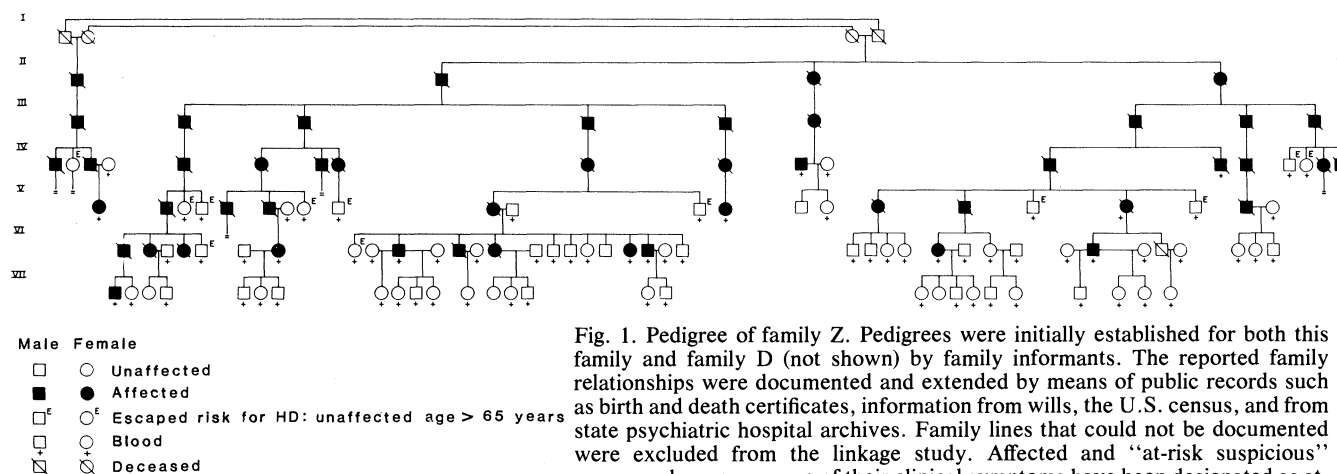


Fig. 1. Pedigree of family Z. Pedigrees were initially established for both this family and family D (not shown) by family informants. The reported family relationships were documented and extended by means of public records such as birth and death certificates, information from wills, the U.S. census, and from state psychiatric hospital archives. Family lines that could not be documented were excluded from the linkage study. Affected and "at-risk suspicious" persons who are unaware of their clinical symptoms have been designated as at-risk on this drawing in order to maintain confidentiality.

HD patients who do not have the mutant gene must wait until age 55 before they can presume that they are unaffected. Many at-risk persons would like to have a test that could provide a reliable indication of their genotype before the age when symptoms are likely to begin (5).

Gusella and co-workers (6) reported close linkage in two families between the HD locus and a DNA fragment, G8, that is located on the short arm of chromosome 4 (7). In fact, at the time of the original report, these sites appeared to be so close that no recombinants were found when two polymorphic restriction sites were analyzed; one recombinant was later detected in analyses of additional members of one of the two families (8). If the same linkage relationship is present in all HD families, one immediate outcome would be a test for HD which could be applied presymptomatically or prenatally. The assumption that there is a single HD locus which is linked to the G8 probe in all affected families would be strengthened if the same linkage relationship were found in HD families from different ethnic groups and in families with differing clinical manifestations. Our data support the existence of a single HD locus, but they suggest that the G8 probe may be further from the HD gene than originally reported, and that further analyses will be necessary before using the G8 probe for presymptomatic testing.

Two large kindreds were ascertained through affected members who attended the HD clinic at Johns Hopkins Hospital. Additional affected members of the kindreds were found during a survey of HD in Maryland, and the various branches of the families were connected by detailed medical genealogy.

Before this linkage study began, we had established close relationships with

the families because of their participation in another project (9). We regularly provided services and genetic counseling to many members of the two families. When we asked the families to participate in this linkage study, we explained that the approximate location of the HD gene had been found in two other families and that we wished to investigate whether the same locus was affected in their families. Informed consent was obtained.

A few at-risk persons were interested in knowing whether they were likely to have HD on the basis of the study results. They were told at the time of blood drawing that we did not know whether the study would give clinically informative results for their family, but that, if it did, they would have the option of knowing their results.

However, we have not yet made test results available to members of these families. Such disclosure must proceed

cautiously and under a research protocol that includes careful explanation of the nature of the test and close follow-up to document and minimize any adverse effects of testing. We are working with the Johns Hopkins Committee on Clinical Investigation to design such a protocol.

The clinical manifestations in the two families were different (Table 1). Family D is an American black family that we traced back five generations to a free black man and his wife, a former slave whom we believe to have been the affected parent. We were not able to establish whether the HD mutation was of African origin or was introduced by racial admixture. The affected members have an early mean age-at-onset of symptoms, and several patients had symptoms before age 20 (juvenile onset). A high proportion of patients have rigidity and akinesia. The disorder begins with a gait disturbance, and severe depressive disorder is rarely observed among affect-

Table 1. Clinical features of families D and Z. "Number affected" represents the affected family members, alive or dead, with available clinical information. A total of 21 affected living members of family D and 16 living affected members of family Z provided DNA for the linkage analysis. Family D and family Z differed in age-at-onset ( $P < 0.001$ ), presence of cases with juvenile onset, effect of paternal transmission on age-at-onset, mode of onset, prevalence of rigidity and akinesia on neurological examination, and the prevalence of an associated manic depressive syndrome. "Paternal transmission effect" refers to the tendency in HD for the affected offspring of affected fathers to have a significantly earlier age-at-onset of symptoms than affected offspring of affected mothers. For family D, the age-at-onset of offspring of affected fathers was significantly earlier than the age-at-onset of offspring of affected mothers ( $P < 0.001$ ); the difference was not significant for family Z ( $P = 0.4$ ). All  $P$  values are based on  $t$ -tests.

Characteristic	Family D	Family Z
Number affected	41	32
Age-at-onset (years) ( $\bar{X} \pm$ standard deviation)	$33 \pm 2.2$	$50 \pm 2.1$
Number juvenile onset	6	0
Mode of onset	Abnormal gait	Psychiatric symptoms
Rigidity or akinesia (symptomatic/total)	5/21	1/15
Percent cases with mania or depression (or both)	2	75
Paternal transmission effect. Age-at-onset (years) if:		
Father affected	25	49
Mother affected	41	52

ed persons. The effect of paternal transmission on age at onset is strong in this family.

Family Z is of German ancestry and was traced back seven generations to their American immigration in about 1750 (Fig. 1). The affected members have a late mean age-at-onset of symptoms, rarely show rigidity and akinesia, often have severe depression, and sometimes show mania in association with the neurological disorder (Table 1). The clinical manifestations of these two families are described in more detail in (9). Blood was analyzed from 64 members of family D and 76 members of family Z. For each family the diagnosis has been confirmed pathologically in two members.

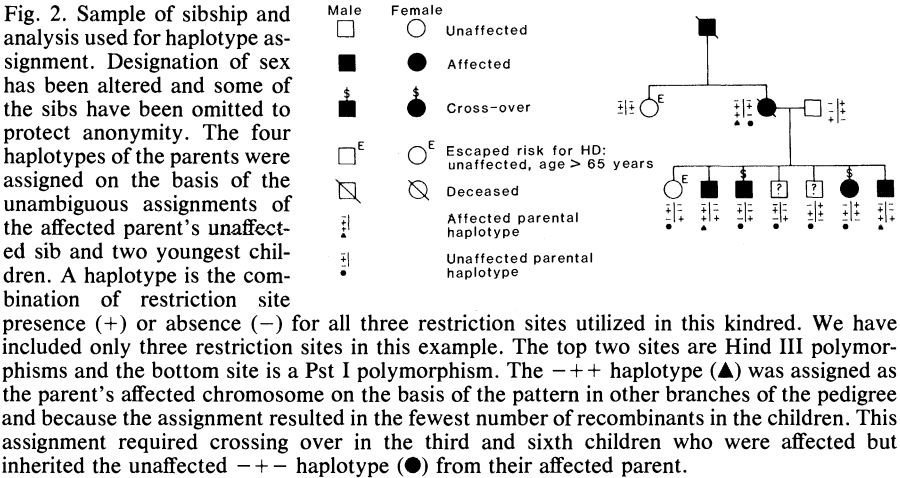
At the time of blood drawing, each family member was examined neurologically and psychiatrically by structured and quantitative methods (9-11). Diagnosis required clear chorea or rigidity and motor impairment in living individuals and reports from medical records or family members for deceased persons. The few individuals who did not meet diagnostic criteria, but who had some neurological abnormalities typical of ear-

ly HD, were called "at-risk suspicious" and were omitted from the computation of linkage results.

High molecular weight DNA was isolated from leukocytes of each subject (12). Hind III, Pst I, and Eco RI restriction site polymorphisms adjacent to the G8 probe were analyzed in studies of duplicate samples. Two polymorphic restriction sites are detected by Hind III (6), one is detected by Pst I (13), and two are detected by Eco RI (13). For most subjects we used only the first Eco RI site. The particular haplotype of each individual for the four or five polymorphic sites was determined by analysis of the sites in various family members. An example of the methods used for haplotype assignment for a sibship in one of the families is shown in Fig. 2. Some of the siblings have been omitted and the sex of some individuals has been reversed to preserve anonymity. Definitive haplotypes could be assigned in more than 95 percent of the individuals, and several could be inferred in deceased parents. Approximately 95 percent of individuals were heterozygous at one or more sites.

Table 2. Accuracy of hypothetical counseling by linkage analysis for HD in families D and Z. If the reported linkage analyses had been used for counseling in these two HD families, 7 of the 60 persons who could be assigned unambiguous haplotypes would have been given incorrect information about their genotype (about 12 percent). These persons were equally distributed between the two families. Three of the elderly escapees had the "affected" haplotype for their families. In two of these three cases (both from family Z), an insufficient number of close relatives were living to be sure that the affected haplotype had not come from the unaffected parent. However, the affected haplotype is rare (0.07), and we gave each of these two escapees credit for half a crossover. The seven cases represent the minimum number of recombinants; an additional three affected persons were probably crossovers, but were excluded for reasons described in the text.

	Counseled	
	Probably affected	Probably unaffected
Actually affected	33	5
Actually unaffected at ≥ age 55 for family D and at ≥ age 65 for family Z	2	20



The paternity reported by the families was checked with 16 serum and red blood cell markers. While no nonpaternity was thus detected, the odds of detecting nonpaternity were low in some sibships. Therefore, in those sibships with apparent recombinants between the G8 probe and the HD locus, additional checks were made with the Eco RI polymorphism detected by the G8 probe (13), with a probe for the growth hormone gene (14), and with the highly polymorphic DNA probe, D1451 (15). This latter probe is a 16-kilobase DNA fragment from the long arm of chromosome 14 and detects eight common alleles. Nonpaternity was detected in one apparent recombinant, and the individual was excluded from the genetic analysis.

The logarithms of the likelihood ratio for linkage at various recombination fractions between the G8 probe and the HD locus, or "lod scores," were computed by the method of maximum likelihood and the computer program LIPED (16). Haplotype frequencies for family Z were estimated from the proportion of the different haplotypes of the 16 unrelated spouses. For family D, estimates of haplotype frequencies were based on data from 6 in-laws and 23 other unrelated American blacks. In these 29 persons the frequency estimate of the affected haplotype was 0.13. Because the frequency estimate for the haplotype associated with HD in family Z was low in spouses (0.07) and was based on a small number of subjects, lod scores were also computed with the frequency of this haplotype set at twice its estimated value. The peak lod occurred at the same recombination fraction when calculated with both estimates, although the magnitude of the lod was somewhat lower when the higher estimate was used. For family Z, the age-at-onset distribution used in the calculations for persons still at risk was taken from Pericak-Vance *et al.* (17). For family D, where the age-at-onset was significantly earlier, we used the age-at-onset mean and distribution derived from affected members.

The peak lod score for family Z was 5.70 and occurred at a recombination fraction of 7 percent. For family D the peak lod score was 7.89 and occurred at a recombination fraction of 6 percent. When both families were combined, the peak lod score, 13.59, occurred at a recombination fraction of 6 percent, with a 95 percent confidence interval between 0 and 12 percent. This estimate of recombination is the most conservative one that is compatible with our data; two other possible recombinants were deleted from the analysis of pedigree D

because of inadequate genealogical documentation: one because of nonpaternity (mother was the affected parent) mentioned above and another because we could not document the reported grandparental relationship. A recombinant found in a distant branch of family Z (shown at the left end of Fig. 1) was not included because of the complexity of computer entry of such a distant relative; she would have contributed little to the recombination fraction either way.

Our results confirm linkage between the HD locus and the G8 probe in two additional families. These families, both with pathologically confirmed HD cases, differ in ethnicity, age-at-onset distribution, presence of juvenile cases, neurological manifestations, and presence and type of psychiatric disorder. Finding linkage to G8 in these families supports the hypothesis that all families with the HD phenotype have a mutation at the same chromosome-4 locus linked to G8. In fact, even though our estimate of the recombination fraction between the HD locus and the G8 probe is greater than that previously reported (6), the 95 percent confidence intervals of the two sets of data overlap (0 to 12 percent as compared to 0 to 6 percent). Furthermore, our findings suggest that phenotypic differences seen in the two families may be due either to allelic differences or to influences from unlinked loci.

By means of three endonucleases that detect four or five polymorphic restriction sites, the proportion of family members who could be assigned unambiguous haplotypes increased, thus improving the utility of the linkage analysis (18). This increase in the proportion of persons with unambiguous haplotype assignments accounts for some of the difference between the estimate of the recombination fraction for families D and Z and that in the original report (6).

If members of these two families had undergone genetic counseling with the G8 probe results, there would have been a 12 percent error rate in counseling of genotype assignment (Table 2). This finding and the remaining uncertainty about the universality of linkage in all HD families (this report brings the number of reported kindreds to only four) suggests that further studies are needed before applying G8 linkage studies to clinical practice.

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## Diversity of Circumsporozoite Antigen Genes from Two Strains of the Malarial Parasite *Plasmodium knowlesi*

**Abstract.** *The complete nucleotide sequence of the coding region of the circumsporozoite antigen gene (CS gene) of the Nuri strain of the malarial parasite Plasmodium knowlesi is presented. The gene from the Nuri strain exhibits a novel form of sequence diversity when compared to the CS gene from the H strain. Instead of the 12 tandem repeating 36-base pair units of the H strain, the Nuri strain contains 16 tandem repeating 27-base pair units of a different nucleotide sequence that encodes a different repeating peptide. In contrast, the 5' and 3' coding and noncoding sequences flanking the repeats are 98 percent conserved in both strains.*

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The gene for the circumsporozoite antigen (CS gene) of the H strain of the simian malarial parasite *Plasmodium knowlesi* has recently been cloned and sequenced (1). A remarkable feature of this gene is the presence of 12 tandemly repeating 36-base pair (bp) units in the middle of the coding sequence. This gene encodes a parasite surface protein with approximately 40 percent of the polypeptide chain consisting of a tandemly repeating 12-amino acid peptide. Since then, a number of surface antigens from the sporozoite and merozoite stages of different malarial parasites (*P. falciparum*, *P. cynomolgi*, and *P. lophurae*) have been cloned, and each of the surface antigen genes has been found to contain tandemly repeating units (2-7). We now present the complete nucleotide sequence of the CS gene of another *P. knowlesi* strain, the Nuri strain (Fig. 1). The gene from the Nuri strain exhibits a

different nucleotide repeat unit than that from the H-strain gene; the 5' and 3' coding and noncoding sequences flanking the repeat units are the same in both strains. Conserved sequences flanking variable repeat regions have recently been reported for S-antigen genes from two isolates from *P. falciparum* (8).

To isolate the Nuri strain CS gene, we constructed a  $\lambda$ gt11 (9) library with blood-form DNA randomly cleaved with DNase I to an average size of 1000 bp (Fig. 2). Using as a probe a 1.6-kilobase pair (kb), Aha III-restriction endonuclease fragment that contained the CS gene of the H strain (1) and was labeled with  $^{32}$ P, we isolated and plaque-purified 11 positive Nuri strain clones from approximately 120,000 plaques. A restriction-endonuclease cleavage map of the Nuri strain clones was constructed and compared with that of the H strain DNA (Fig. 2). The cleavage sites appeared to be identical for 2.5 kb in both strains. Four  $\lambda$ gt11 clones,  $\lambda$ KN5,  $\lambda$ KN6,  $\lambda$ KN7, and  $\lambda$ KN8, were used to sequence the Nuri strain CS gene. The nucleotide sequence of the CS gene was established by the Sanger dideoxy chain-termination method (10) after the inserts were subcloned into M13 sequencing vectors (11).

The complete nucleotide sequence and the deduced amino acid sequence of the Nuri strain CS gene is shown in Fig. 2. For the regions 5' and 3' to the repeating units, the sequence in the Nuri strain is 98 percent identical to that in the H strain. The diversity of the sequence of repeating units, however, is striking.