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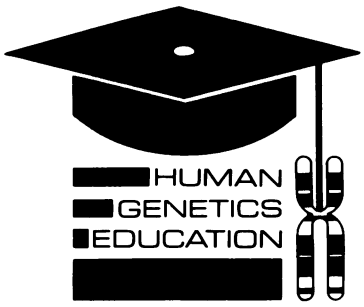
FEATURE ARTICLE
The Paradigm of Huntington Disease



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> I recall it as vividly as though it had occurred but yesterday. It made a most enduring impression upon my boyish mind which was my very first impulse to choosing chorea as my virgin contribution to medical lore. Driving with my father through a wooded road leading from East Hampton to Amagansett we suddenly came upon two women, mother and daughter, both tall and thin, almost cadaverous, both bowing, twisting, grimacing. I stared in wonderment, almost in fear. What could it mean? My father paused to speak with them and we passed on. Then my Gamaliel-like instruction began; my medical education had its inception. From this point on, my interest in the disease has never wholly ceased. [George Huntington, at 59, recalling how at the age of 8 years he first saw Huntington disease while traveling with his physician father on his professional rounds in 1858].

Introduction

Huntington disease (HD) is an incurable late-onset autosomal dominant disorder characterized by degeneration of the nervous system. Characteristic features of HD include involuntary choreic movements (in the United Kingdom, the disorder is referred to as Huntington chorea), the impairment of cognitive functions, and behavioral changes. Each child of an affected person has a 50% chance of inheriting the defective gene. The penetrance of the defective allele is 100%; thus, all individuals who inherit it manifest symptoms of the disease and ultimately succumb to its effects, unless they die of other causes. Though relatively rare (about 4–8 cases/ 100,000 people), it is among the most mysterious and peculiarly distressing of genetic disorders. Part of the mystery lies in how the HD gene can cause the programmed death of a specific subset of brain cells.

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The HD gene was localized to chromosome 4 in 1983 by Gusella et al. (1983). This identification was made possible by the use of an RFLP, a DNA marker that was found to be closely linked to the HD locus. The RFLP is one of the most powerful tools available today to detect defective alleles.

The Gusella et al. paper was seminal. It was the first in a series of important papers that have been steadily unraveling the mystery of the gene that causes HD. That paper and the several that have since followed illustrate many important genetic concepts, one of which is the association between a phenotype and a specific genotype. There is a great deal of interest today in the links between the genotype and neurological function. With HD we have a single-gene locus that when mutated causes a neurodegenerative disorder, a disorder associated with a specific subset of brain cells (basal ganglia and cerebral cortex). HD illustrates other things as well. It exemplifies the integrative approach to genetics by bringing to bear on a specific problem the tools of molecular, classical, and population genetics. For this reason and others, HD can be used with great effectiveness as a key pedagogical tool in human genetics courses in medical schools, colleges, and universities.

The Genetics of HD

HD is inherited as an autosomal dominant trait. The enormous Venezuelan kindred along the shores of Lake Maracaibo and the smaller American kindred illustrate the key features of autosomal dominant inheritance, as seen in figure 1.

But HD illustrates much more than just simple autosomal dominant inheritance. Like many other autosomal dominant traits, it is a late-onset pleiotropic disorder exhibiting variable expressivity in both the age at symptom onset (2–80 years) and degree of cognitive and emotional disorders. Furthermore, we find that there is no evidence for genetic heterogeneity, since all

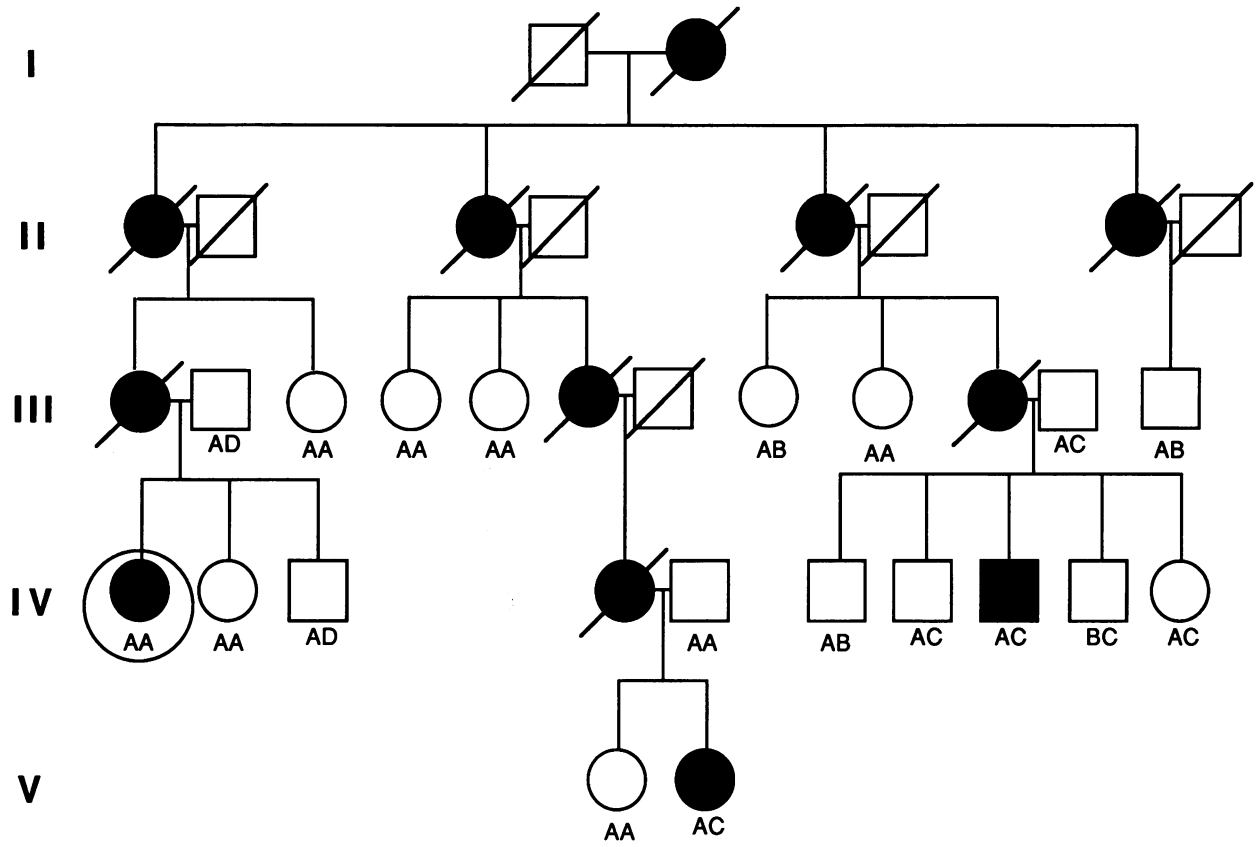

Figure 1 A portion of the Venezuelan HD pedigree. Deceased individuals have a diagonal slash through the symbol. This pedigree illustrates autosomal dominant inheritance. The letters under the symbols refer to the D4S10 molecular marker haplotypes found in each person. In the Venezuelan pedigree, haplotype C segregates with the HD allele. The person circled represents a recombination event between the D4S10 locus and the HD locus (after Gusella 1984).

families studied who express the trait carry mutations at the same locus on chromosome 4 (Folstein et al. 1985; Haines et al. 1986).

HD allows us to discuss another important genetic concept, that of multiple allelism. While all studies to date indicate that there is a single locus responsible for HD, given the diverse clinical features of the disease in different families, we may well be looking at different allelic mutations at the same HD locus. A recent study (Wolff et al. 1989) suggests that a new mutation occurred at the HD locus in a large kindred. A woman developed the symptoms of HD at age 36 years, and they progressed for more than 14 years. Her parents, who lived into their eighties, did not develop any symptoms; nor did any of her 15 living siblings, 13 of whom were older. The most reasonable interpretation of this pedigree is that a new mutation arose at the HD locus. If this interpretation is correct, then this mutation is a prime candidate for a new HD allele. Other interpretations of the clinical variability are, of course, possible. For example, the variable expressivity of HD may be a consequence of differences in genetic background.

Also of interest here is the fact that HD is the only genetically determined human trait known that shows true Mendelian-dominant inheritance: homozygotes and heterozygotes for the HD allele are phenotypically indistinguishable. For most human dominant traits, the homozygote is so severely affected that it does not usually survive to birth. An example of this is achondroplasia. That HD behaved as a true Mendelian dominant was determined by identifying unions between individuals in the Venezuelan kindred who developed the symptoms for HD. The offspring from these unions were examined, and probable homozygotes were identified on the basis of DNA markers. In one such mating that produced 14 children, four children were identified as

probable HD homozygotes (six were HD heterozygotes, and four were not carrying the HD allele; this approximates a classic 1:2:1 Mendelian ratio). The probability that none of the four is homozygous for HD is very small (about 1/25,000), suggesting that homozygosity for the HD allele does indeed exist in this family and that it is not lethal in utero. There appear to be no phenotypic differences, either in age at onset, symptoms, or progression of illness, between HD homozygotes and HD heterozygotes (Wexler et al. 1987).

The Molecular Genetics of HD

In 1980, Botstein et al. (1980) published a theoretical paper on how RFLPs could be used to map gene loci. RFLPs would be especially valuable tools in mapping gene loci—such as the HD locus, the cystic fibrosis (CF) locus, and others—whose products have yet to be identified. This was a profound leap forward in thinking about gene mapping. In its most intriguing form, gene mapping with RFLPs involves linkage analysis of two gene loci, both of which encode unknown products. One locus in the HD analysis is a segment of DNA with no known function, detectable as being polymorphic for restriction-enzyme sites. The other locus is the HD locus, whose cellular function continues to elude us. These genetic markers are traced together in families segregating for the HD allele. If the two loci are linked, they will cosegregate; if unlinked, they will assort independently.

The HD locus was the first autosomal locus to be mapped using an RFLP (Duchenne muscular dystrophy, an X-linked locus, was mapped in 1982 using RFLPs). This remarkable feat was achieved by discovering a DNA marker that cosegregated with HD. Though testing hundreds of probes that detect RFLPs could have been necessary based on the probability of finding close linkage to HD, Gusella and his colleagues fortuitously found what they were looking for among the first 12 tested. They discovered that the G8 sequence (now renamed D4S10—D for DNA, 4 for chromosome 4, S for segment, and 10 for the order of identification of probes mapping to chromosome 4) cosegregated with HD.

RFLP analysis illustrates several important features of recombinant DNA technology and how it can be used in a very positive way. The Gusella et al. (1983) paper has a clear discussion of RFLPs. However, this topic can be greatly expanded by the inclusion of other readings (Gusella 1986).

It is valuable to contrast the use of RFLPs in the analysis of genes with unknown function, such as the HD gene, with the use of RFLPs in the analysis of genes with known function, such as the beta-globin gene. For example, the mutation for sickle cell anemia is found in the sixth codon of the first exon of the beta gene; this same mutation also destroys the recognition site for the restriction enzyme *Mst*II. Thus, the mutation alters both a restriction site and the site coding for an amino acid (Change and Kan 1982). With the D4S10 probe, we do not identify sequence variability in the HD gene. As a matter of fact, the D4S10 sequence is 4–5 map units away from the HD locus. In the sickle cell case, we can identify the beta-globin gene mutation with essentially 100% accuracy because the mutational change detected as an RFLP is the one causing the disease. With HD, identification of the HD allele is accomplished only by having the two separate variants linked—and thus is complicated by crossing-over between HD and D4S10.

The D4S10 Allele Frequencies in the Human Population

Population genetics, often given minimal coverage in human and medical genetics courses, can be illustrated clearly using the four different D4S10 haplotypes available. Each haplotype, A, B, C, and D, represents a differ-

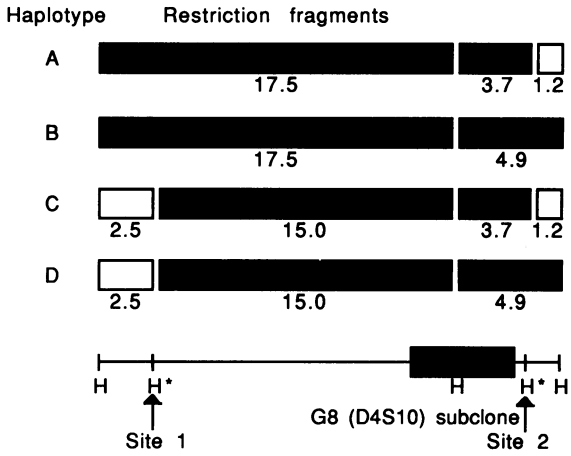

Figure 2 Haplotypes at the G8 (D4S10) locus. There are two polymorphic *Hin*dIII sites (site 1 and site 2) at this locus. At each site there are two possible alleles: the presence of the site (+) or the absence of the site (−). The combination of alleles at these sites is referred to as a haplotype. At the G8 (D4S10) locus there are four haplotypes: A, B, C, and D. The part of the G8 locus that serves as the probe is shown at the bottom, and the fragments it detects are shown as shaded. Those segments that are unshaded are not picked up by the probe because they do not overlap it (after Gusella 1984).

ent pattern of cleavage by the restriction enzyme *Hin*dIII (fig. 2). Each pattern can be viewed as an allele comprised of the presence (+) or absence (−) of the polymorphic *Hin*dIII restriction sites 1 and 2 at the D4S10 locus. Gusella et al. (1983) determined the frequencies of these alleles in a sample of 23 unrelated people in North America. Once these frequencies were determined, they calculated the four expected combinations, i.e., +−, −+, ++, and −−, for the two variable sites. Are the combinations of sites random for their frequencies, or is there some preferential association of sites (e.g., is there is an advantage to having ++)? In other words, is there evidence for linkage disequilibrium? No evidence for linkage disequilibrium was found, but the calculations allowing this conclusion are illustrative of the very important principle of the Hardy-Weinberg equilibrium.

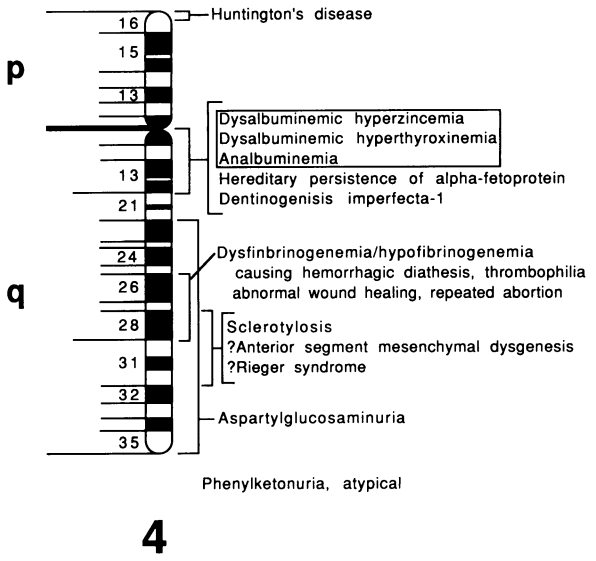

Figure 3 Chromosome 4. The boxed entities are allelic disorders (after McKusick 1987).

The Integrative Strategy of Mapping the HD Gene

Mapping the HD locus required more than finding a DNA marker that cosegregated with HD. The D4S10 locus did indeed segregate with HD, but which autosome carried this DNA marker? The identification of the specific autosome that carried D4S10—and thus HD—was achieved using somatic cell genetics, one of the most important advances in the field of human genetics.

Gusella et al. (1983) located the autosome that carries the D4S10 locus by looking at various human-mouse cell hybrid lines. Only those cell lines that carried human chromosome 4 had the D4S10 sequence. This technique has been extremely important in localizing genes whose function can be detected in the hybrid cell line. Here, however, we have an instance where a gene locus is identified on a specific chromosome even though it produces no known product.

The HD locus was regionally localized on chromosome 4 when it was discovered that patients with Wolf-Hirschhorn syndrome, a birth defect caused by the deletion of part of the tip of the short arm of chromosome 4, were also missing the D4S10 sequence (Gusella et al. 1985). This localized the HD locus to the tip of chromosome 4 (fig. 3).

The D4S10 locus was localized more precisely using the technique of in situ hybridization (Magenis et al. 1986). This powerful technique uses labeled D4S10 DNA as a probe to bind directly onto banded chromosomes held in place on a slide. The labeled probe binds to its homologous sequence on a specific chromosome. Using four patients carrying different deletions in the terminal region of 4p, Magenis and her colleagues found that D4S10 localized to the distal half of band p16.1 of chromosome 4.

With D4S10—and, by inference, HD—localized to the tip of chromosome 4, the task now becomes one of analyzing the linkage relationship of HD to D4S10. How close is D4S10 to the HD locus? Most students will be familiar with the traditional types of linkage analysis: the classic two- and three-point crosses. The same principles that apply to those analyses also apply to the mapping strategy for HD, but with important embellishments. With HD, DNA markers identified as RFLPs for the D4S10 locus are used in the linkage analysis. The lod score analysis (logarithm of the odds), a powerful classical tool in human linkage studies, was effectively used in the linkage analysis of the D4S10 locus and the HD locus. Lod score analysis is a mathematical statement of the probability of linkage between two loci at some specified rate of recombination. The distance between two linked loci is expressed as a recombination frequency. The establishment of linkage is determined by generating a lod score. The lod score is calculated by evaluating the probability that an observed pattern of allele segregation in a family occurred as a consequence of independent assortment or linkage between two loci. While a detailed discussion of the lod score technique and how it provides information about

linkage may be beyond the scope of many human and medical genetics courses, a general description of it emphasizes the importance of probability and traditional mapping techniques in pedigree analysis (see Muench 1988).

With HD, it was originally estimated that D4S10 was 0 map units away from the HD locus, because no recombinants were found. In other words, the exciting possibility was raised that the D4S10 sequence was part of the HD locus. However, as linkage studies were extended to additional kindred members, some recombinants were found (fig. 1), but the linkage remained close. Today lod score estimates suggest that D4S10 and HD are about 4 map units apart. RFLPs closer to HD than D4S10 have now been identified (Wasmuth et al. 1988; Robbins et al. 1989). Two of these RFLPs (D4S95 and D4S90) lie between D4S10 and the HD locus. The sequence of markers is now thought to be D4S10–D4S95–D4S90–HD–telomere. As we find markers that map closer and closer to HD, we approach the prospect of having a marker that includes part of the HD gene sequence. An interesting problem for discussion at this point might be this: if the difference between a normal and mutant HD allele is a single base pair, or perhaps a few base pairs, how will we know when we have in fact isolated or entered the HD gene?

The mapping strategies used to locate the HD locus are important. They reflect strategies used successfully in the mapping of several other gene loci. These strategies include traditional techniques, molecular techniques, and somatic cell techniques.

The Personal and Ethical Dilemmas of HD

Human genetics is an intensely personal discipline, perhaps more so than any other scientific discipline. It has as its goal the characterization of our genetic being. Identifying flaws in that genetic material requires the appropriate scientific methodology as well as attention to the personal and ethical considerations of the individuals and families involved. In medical genetics, no topic is charged with as much emotion or controversy as the subject of prediction for the person at risk for developing HD.

In families where HD is segregating, several important issues need to be addressed. One is the accuracy of the presymptomatic testing procedure itself. There is a possibility of crossing-over between HD and the molecular marker, which means that the probability of carrying or not carrying the allele is approximately 95% rather than 100%. This complicates predictive testing. The complications have become even greater now that we know recombination can occur between restriction stites *within* the D4S10 locus (Curtis et al. 1989). The accuracy of presymptomatic testing can be increased by using multiple markers for the D4S10 locus and by using other RFLP loci as well.

A second issue is how individuals who are at 50% risk for developing HD respond to the knowledge that DNA probes can now be used to identify, with a high degree of accuracy, the defective allele. On the basis of recent studies, it is estimated that about two-thirds of the people at risk for HD intend to seek predictive testing, though the percentage of people at risk who ultimately receive the testing is much less. A negative test result reduces anxiety about developing HD and clarifies the future for the person. A positive result in a person poorly equipped to handle this information can be devastating. For people at risk for HD, a minimum of 4 h of counseling and 8 h of psychological evaluation are typically involved in the procedure before the communication of test results. In a recent study of 15 people at risk for HD (Meissen et al. 1988), four were identified as probably having HD. The four individuals identified as having HD understandably experienced depression but were not suicidal. The sample was small, and much more data are needed in this area. Predictive testing for HD when there is no cure or preventative therapy is very difficult and requires great skill. Such predictive testing raises several ethical issues that are fruitful areas of discussion (Shaw 1987).

Conclusion

HD illustrates several important genetic concepts. These concepts include segregation and the lack of independent assortment as seen in pedigree analysis, autosomal dominant inheritance patterns, late-onset disorders, multiple allelism, variable expressivity, pleiotropism, recombinant-DNA techniques, molecular mapping using RFLPs, Hardy-Weinberg equilibrium, linkage disequilibrium, somatic cell genetics, lod score analysis, probability, traditional mapping techniques, the relationship between a phenotype and a genotype, and the many personal, ethical, and societal dilemmas that can be associated with human genetic diseases. HD is extremely valuable as a teaching instrument in human and medical genetics courses.

Acknowledgments

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Appendix

Editor's Note: Supplemental Videotapes Available

Several related videotapes of excellent quality exist, and they are strongly recommended for use in any educational setting where readers believe the contents of this article appropriate. Each is available in ½" VHS by contacting

Huntington's Disease Society of America
140 West 22d Street
New York, NY 10011
(212) 242–1968.

- "NOVA" program originally broadcast November 12, 1985, as part of "The Genetic Gamble" (HD segment approximately 15 min). Visits to the Venezuelan families with Nancy Wexler, describes HD, explains the pedigree analysis and DNA marker work of James Gusella, and has an excellent description of how presymptomatic diagnosis is accomplished through Nancy Wexler's use of dominos to exemplify linkage analysis.

- "60 Minutes" with Diane Sawyer, originally broadcast November 25, 1986 (14 min). Also shows the Venezuelan families and describes HD, but emphasis is placed on the difficulty many at risk have in making the choice of whether to use the presymptomatic test. The Wexler family discusses this process of decision making and considers the risk/reward ratio as a critical determinant.

- "West 57th Street" with Meredith Vieira, originally broadcast June 2, 1987. (12 min). Discusses how the Carrother's family has coped with the father and a daughter dying from HD and how one of the two children decided to have the presymptomatic test. Describes the extensive process involved in using the test, and the need for greater consideration of the consequences of this and many other genetic tests becoming available.

- "NOVA" program "Confronting the Killer Gene," originally broadcast March 28, 1989. (57 min). Focuses on four individuals from HD families, one a child who is already affected, the other three being individuals at risk, including Arlo Guthrie and Nancy Wexler. Each discusses how he or she came to a decision as to whether to use the presymptomatic test. Also, James Gusella is interviewed, and the Venezuelan families are shown.

The 1985 and 1989 "NOVA" programs can also be purchased in ½" VHS for $99.00 and $250.00 respectively from

Cornet/MTI Films
108 Wilmot Road
Deerfield, IL 60015
(800) 621–2131

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