Huntington's Disease Gene Located

A multidisciplinary team has found a marker near the gene, which is scientifically exciting but which raises ethical problems associated with testing those at risk

In 1979, the Hereditary Disease Foundation held a workshop on using recombinant DNA mapping to find the Huntington's disease gene. "A lot of people were skeptical—it sounded like science fiction," says Nancy Wexler, the president of the foundation. No one had any idea where the gene might be and no one had any way of recognizing the gene if they found it. And there are 46 human chromosomes and 3 billion base pairs of DNA to search through. Still, Wexler recalls, "Everything else looked kind of bleak, so we figured why not try?"

Now, a collaborative group of investigators* reports that they are almost there. They have found a restriction enzyme marker—a piece of DNA that can be pinpointed with recombinant DNA techniques—that is so close to the Huntington's disease gene that its presence can be used as an indicator for the gene. (They report their findings in the 17 November issue of *Nature*.)

"This has *radically* changed the face of Huntington's disease research," says Wexler. "All of us are ecstatic." What the finding means is that scientists now know exactly where to look for the gene or genes that cause the disease. It is only a matter of time until they isolate it. Once the gene is isolated, it should be possible to learn what it does and, perhaps, prevent it from being expressed or prevent it from causing the devastating Huntington's disease symptoms even if it is expressed.

For now, however, the finding means that a large number of persons at risk for getting Huntington's disease will be able to learn whether they will, in fact, get it—a prediagnostic test that for some is a mixed blessing and that certainly raises a host of ethical questions.

Huntington's disease, which killed folk singer Woody Guthrie, starts insidiously, usually in persons between the ages of 35 and 45, although it can occur anywhere between age 2 and 80. The first sign is tiny abnormal movements, ticks, and clumsiness. "Gradually," says Wexler, "the entire body is encompassed by adventitious movements. The trunk is writhing and the face is twisting. The full-fledged Huntington's patient is very dramatic to look at. There is also an intellectual decline." Going along with and often preceding these symptoms are profound emotional disturbances that frequently are misdiagnosed as manic depression or schizophrenia. The disease progresses for 10 to 20 years until the patient dies.

Although no one knows what causes the disease, over the years it has become clear that it behaves as if it is controlled by a classic dominant Mendelian gene. In other words, if one parent has Huntington's disease and the other does not, each child has a 50 percent chance of inheriting the disease gene and every child who gets the gene will eventually get the disease. These findings are the reason researchers talk about "the" Huntington's disease gene. It is possible, of course, that the disease is caused by a collection of genes but, if so, they must be so tightly linked that they are always inherited as one.

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For scientists, Huntington's disease is fascinating. Here is a single gene or a tightly linked collection of genes that is expressed usually in mid-life and that has profound effects on mood, motor control, and intellectual functioning. But, says David Housman of the Massachusetts Institute of Technology, who helped initiate the current search for the Huntington's disease gene, those who start to study the disease soon get emotionally involved as well. "A disease like Huntington's really makes you humble, it changes your perspective on science. You're not doing this for your ego. For me, it became an intense personal thing."

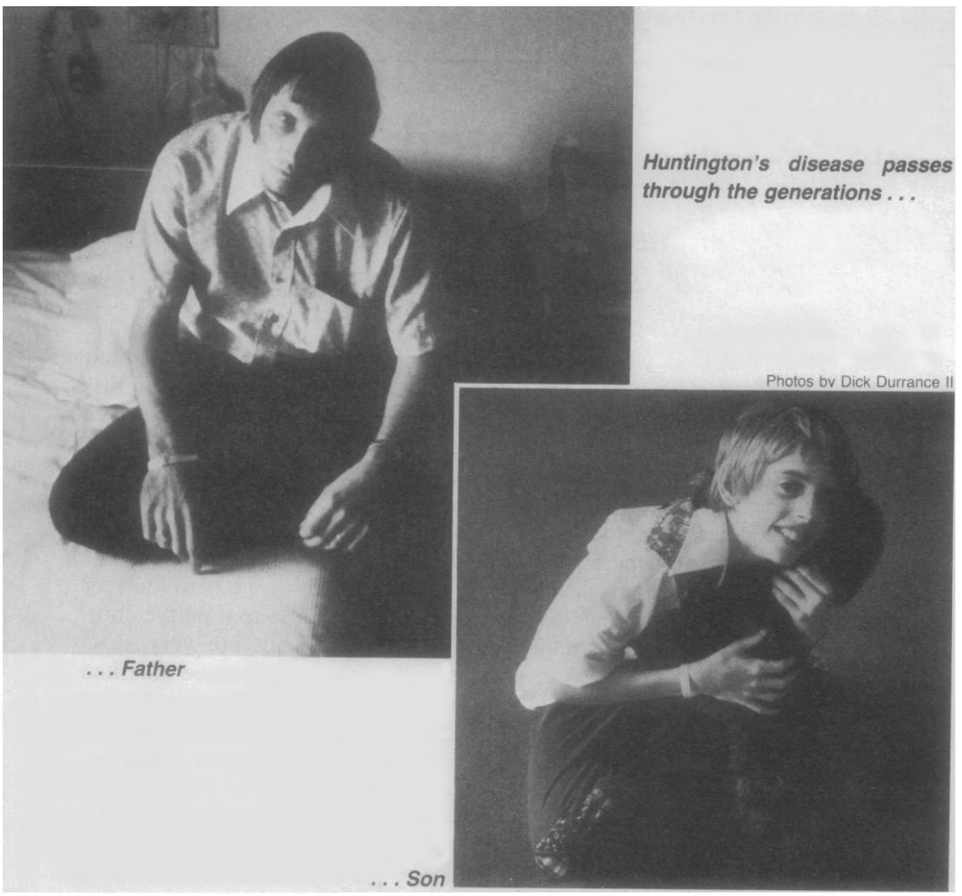
The only hard piece of scientific data that is of value in trying to understand the basic problem of Huntington's disease, however, is the fact that it behaves as if it is controlled by a classic dominant gene. For that reason, says Wexler, it seemed that the best way—the only way—to go about studying the cause of the disease is to find the gene.

One way to find a gene is to look for markers that are close to it on the chromosome. If two genes are very close together, they are likely to be inherited together. If you can see one gene but not the other, you may be able to establish their proximity by looking at pedigrees. For example, if one gene codes for an enzyme in saliva and the other causes a disease, you may be able to establish that every person who inherits the gene for the saliva enzyme also gets the disease, even though the saliva enzyme has nothing to do with the disease. This approach worked for some diseases, such as myotonic dystrophy, but it did not work for Huntington's disease. There simply were not enough protein markers. T. Michael Conneally of Indiana University, who heads one of several research teams that looked for protein markers for the Huntington's disease gene for 12 years, says, "We looked at 30 to 35 markers. It was very frustrating."

A few years ago, molecular biologists found a new way to get gene markers. They discovered that there are variations in the pattern of DNA that are inherited as traits. Some of these variations are at sites where restriction enzymes cut DNA. Each restriction enzyme cuts DNA only at a specific base sequence. If different people have variations in their DNA at these sequences, then when their DNA is cut by these enzymes, there will be different patterns of cutting. The hope was that there would be a correlation between a particular pattern of restriction enzyme cutting and the presence of the Huntington's disease gene.

What was needed was a large number of families with Huntington's disease, or, even better, a large number of interrelated families who carry the gene and tend to have lots of children. In this way, the researchers could follow the inheritance of the gene and—if they were lucky—

*The principal investigators are James Gusella of Massachusetts General Hospital, Nancy Wexler of the Hereditary Disease Foundation in Beverly Hills, P. Michael Conneally of Indiana University Medical Center, Susan L. Naylor of Roswell Park Memorial Institute, and members of the Venezuela Collaborative Huntington's Disease Project.



correlate it with a restriction enzyme marker. A few years ago, the Hereditary Disease Foundation established a roster of American families with Huntington's disease. Families could join if they wished and those who joined supplied data on their family tree and on all aspects of the disease in their family—age of onset, cause of death, clinical syndrome. These data are kept by Conneally and his associates in Indiana and the largest of the families was asked to supply blood and skin samples for the search for the Huntington's disease gene.

An even better source of Huntington's disease families is in Venezuela along the shores of Lake Maracaibo. In the early 1800's, a woman living there got Huntington's disease—most likely, it is thought, because her father was a European sailor or trader who carried the gene. Since then, the woman's ancestors have mostly stayed near Lake Maracaibo and there are now a total of more than 3000 people in her lineage. There are 100 Huntington's disease patients living there now and 1100 children who are at 25 or 50 percent risk of getting the disease. Wexler, who led an interdisciplinary team to Venezuela to get skin and blood samples, family histories, and to do neurological exams, remarks, "The families are large—15 or 18 children is typical—and they are beautiful for genetic studies."

The investigators collected blood and skin from 570 individuals during the course of several visits to Venezuela. Each time, the samples were transported 12 hours by boat to an airport in Maracaibo. From there they were flown to Miami and from Miami they were flown to Boston and the laboratory of James Gusella at the Massachusetts General Hospital. It was Gusella and his colleagues who were to do the restriction enzyme mapping to search for a link with the Huntington's disease gene.

Gusella also was looking at the samples from the American families, and in late summer he thought he saw a weak correlation between a restriction enzyme cut on chromosome 4 and Huntington's disease. The odds were 80 to 1 in favor of linkage, but geneticists look for much better odds than that. Still, it was a good hint and Gusella decided to home in on it. What he needed was more families. He looked at the Venezuelan families and, sure enough, the marker also predicted Huntington's disease in them. By adding these families, he got odds of over 100 million to one that the restriction enzyme marker is linked to the Huntington's disease gene.

Now the researchers know that the Huntington's disease gene is on chromosome 4 and that it is somewhere within an area of several million base pairs—an area large enough to hold about 100 genes. This is an area constituting a few percent of the length of the chromosome.

It is possible, of course, that other Huntington's disease families will have other genes located, perhaps, on other chromosomes and the investigators will be looking for such genes. But Gusella suspects they will not find them, that the gene on chromosome 4 is the only Huntington's disease gene. "The likelihood that it will hold up is good," he says. "There is no case described of a new Huntington's disease mutation and there is also a very clear migration pattern of the gene from western Europe. Probably only one gene is involved."

The immediate clinical consequence of this work is that it will now be possible to tell many people who have Huntington's disease in their families whether they inherited the gene. The test is not yet clinically available, however, and it will not help everyone. A person at risk who wants to know if he has the gene needs, ideally, blood samples from himself, his parents, and his grandparents. As more data on the restriction enzyme correlation are acquired, it should soon become easier to be tested.

But, cautions Conneally, "There will be lots of problems." Some people who watched a parent die of the disease and know they have a 50 percent chance of getting it themselves really do not want to know whether they have the gene. They feel they would not be able to cope with the knowledge. Yet their spouse and children may feel entitled to the information. Or, says Conneally, take the case of a man who is at risk for the disease and does not want to know whether he has the Huntington's disease gene. His wife becomes pregnant and wants amniocentesis to learn whether she is carrying a child with the gene. If she has the prenatal test and the fetus is affected, it means the father is carrying the gene.

"What should we do? This is a major problem and we can't kid ourselves about it," says Conneally. His own institution has already established a committee to draw up guidelines for testing for the Huntington's disease gene. Among other things, they are considering what sort of psychological counseling is necessary before people are tested. Should people have a psychological work-up to see if they are capable of coping with the information? How should the spouses be counseled about the test? "We have to tread very carefully. We can't just set up shop like we're testing people to see if they're Rh positive," Conneally says.

In the meantime, the investigators are being scrupulous about protecting the information they already have on the American and Venezuelan family mem-

bers who are carrying the Huntington's disease gene but do not know it. They have not yet told the American family that they were the ones whose cells were used in this project. And they did not publish all of their data on the Venezuelan family in the *Nature* paper, writing instead, "Although a number of younger at-risk individuals were also analyzed 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."

The next steps scientifically are a bit easier. The first thing to do, says Gusella, is to check more families to see whether there are other Huntington's disease genes elsewhere on the genome. Next, they want to get more restriction enzyme markers, including ones on either side of the gene, that will allow them to zero in on it. What this does, says Housman, is to narrow down the outer limits of where the gene can be. "We may get down to 500,000 base pairs. Then we will work through that trying to figure out what relates to the disease."

But how do you go from a 500,000 base pair region to the isolation of a specific gene when you have no idea what the gene looks like or what it does? "That question is really at the cutting edge of science right now," Housman says. "I can give you five or six ways we can try but I can't give you a simple answer." Basically, however, there are two approaches. Researchers can take a functional approach, asking what kinds of RNA's are coded by the region, then figuring out what proteins are made and asking whether any of these might be associated with Huntington's disease. Or they can take a genetic approach, precisely comparing the DNA in this region and looking for a difference between unaffected persons and carriers of the Huntington's disease gene. In any event, the investigators expect that it will take years before they actually isolate the gene and find out what it does.

But the difficult work ahead of them in no way diminishes the researchers' enthusiasm. This is the first time anyone has used restriction enzyme markers to locate a gene that could have been anywhere on the genome. Always before they at least knew on which chromosome to begin looking. And although they always thought the restriction enzyme techniques would eventually lead them to the Huntington's disease gene, everyone thought that it would take at least 5 or 10 more years. "It is shocking but it's wonderful," Wexler says.

—GINA KOLATA

Viroid Origin in Jumping Genes?

Viroids are the smallest known infectious agents, which so far have been shown to occur only in plants. They are single-stranded RNA molecules and typically are from 270 to 380 nucleotides long; this is at least three orders of magnitude smaller than even the most diminutive virus. Unlike viruses, viroids are naked: the RNA strand is not encapsulated in a protein coat. Despite many attempts, no one has found a viroid-encoded protein: the genome, it seems, might not be translated at all. Viroids, clearly, are mysterious beasts. Recent analysis of nucleotide sequences of one group of viroids has, however, given a possible glimpse into the origin of these enigmatic pathogens. Theodore Diener and his colleagues at the Plant Protection Institute, Beltsville, Maryland, report features in viroid sequences that echo structural aspects of transposable genetic elements.*

In addition to encoding certain essential aspects of their insertion into and excision from their host genome, transposable elements often carry other potentially active genes. Because of many structural similarities between transposable elements and retroviruses, in particular certain sets of direct and inverted repeat sequences, there is now wide agreement that these RNA viruses evolved from mobile elements, presumably, among other things, by adding groups of cellular genes to the mobile unit. The life cycle of retroviruses includes reverse transcription into DNA, which becomes integrated into the host genome. By contrast, viroids might have derived from transposable elements by the loss of internal coding regions and the deletion of sections of the so-called long terminal repeats (LTR's). Moreover, viroids do not pass through a DNA stage in their life cycle: they are replicated directly as RNA, by an as yet to be determined host enzyme, and are not integrated into the host genome.

When Michael Kiefer sequenced tomato planta macho viroid and tomato apical stunt viroid earlier this year, he saw that they were related to three others that formed the potato spindle tuber viroid group. Kiefer, together with Diener and Robert Owens, was therefore able to look for sequence patterns within the group and with other organisms. One stretch of 28 nucleotides, located around position 100, is identical between the five viroids, and a second stretch, around position 320, is virtually so. Because the single RNA strand of viroids is often covalently linked as a closed circle, the overall structure may conform to a stiff "rod," which is held together by extensive base pairing so typical of viroid sequences. This arrangement happens to bring the two highly conserved regions opposite one another, forming what has become known as the conserved center.

The significance of the conserved center is that in the upper strand the sequences are formed from two more or less complete direct repeats, which are flanked by somewhat incomplete inverted repeats; the dinucleotides CA and UG also flank the direct repeats. These patterns, which are echoed imperfectly in the lower strand of the viroid loop, are very similar to structural features of LTR's in transposable elements but with certain sections deleted. In addition to the repeats in the central section of the viroids, there appear to be many remnants of inverted repeats scattered throughout the body of the molecule.

Another feature common to the potato spindle viroids is a stretch of 11 to 18 uninterrupted purine bases centered on position 60. Such a signature is reminiscent of the putative signal site for reverse transcriptase activity in retroviruses. Its presence in viroids, which apparently do not make DNA copies of themselves, might bespeak a common origin with retroviruses. The site might have become modified for initiating RNA synthesis.

If viroids do indeed derive from transposable elements one would expect to see somewhere in the host genomes at least some sequences similar to those of the viroids. So far no one has found such sequences, but searches have been made in the commercially important plants in which viroids cause pathologies rather than in wild plants in which they originated. Diener plans soon to shift the search to these wild plants.—ROGER LEWIN

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