CLOSING IN ON A KILLER GENE

Genetic detectives are using recombinant DNA to solve the mystery of Huntington's disease, but victims may not want to know the answer

BY GINA KOLATA

Geneticist Peter Harper and social worker Audrey Tyler of the Welsh National School of Medicine traveled through south Wales in the 1970s, interviewing people whose parents or relatives were victims of Huntington's disease and who might have inherited a gene that would give them the disorder, too. During the interviews, the researchers asked a hypothetical question. If there were a test to tell if you have the gene, would you want to take it? More than half said no. They knew all too well the consequences of having the gene: you are doomed. There is no treatment for Huntington's disease, and it is always fatal.

The disease, moreover, punishes its victims cruelly before it kills, depriving them of control of their bodies and the use of their minds. It is better, many potential victims think, to live with the hope that they have not inherited the gene than to take the chance of finding out that they have it. In the United States alone there are at least 20,000 diagnosed victims of the disease, and another 100,000 who may have the gene.

Within just a few years—far sooner than scientists had expected—there will be a test for the Huntington's disease gene, and the question asked in Wales will no longer be hypothetical. A group of researchers has found a marker for the gene—a section of DNA that is on the same chromosome as the gene, is close to it, and seems to be inherited with it. By testing for the presence of the marker, doctors will be able to determine if the gene is there, too.

The discovery was a remarkable feat—and a surprise. When the search for the gene was initiated in 1979 by the Hereditary Disease Foundation in Santa
UNWINDING THE DNA SPIRAL

1. A human cell has a nucleus with 46 chromosomes that contain about 100,000 genes and at least 3 billion base pairs, the letters of the DNA code.

2. The chromosomes, 23 of them contributed by the mother, 23 by the father, consist of DNA and protein.

3. DNA, tightly coiled, can be released from the chromosome with a chemical wash and unraveled to form a long strand.

4. Segments of DNA interspersed among the genes can be used as markers to seek out specific genes.

5. The precise function of many segments of DNA remains a mystery.

Monica, California, and the National Institute for Neurological and Communicative Disorders and Stroke (NINCDS) in Bethesda, Maryland, scientists enlisted in the hunt did not expect to find the marker, let alone the gene, for at least a decade. The early success, says Nancy Wexler, president of the foundation, "has left us stunned."

The discovery has made science history. It marks the first time that scientists have used recombinant DNA technology to find the approximate location of a gene without having a clue beforehand as to where in the complex human genetic structure the elusive gene lies. The scope of the search was immense: it covered 46 chromosomes, about 100,000 genes, and a total of 3 billion base pairs—the rungs in the spiraling ladders of DNA. Yet all these elements are packed into the nucleus of a human cell, a space so small that researchers must work virtually blind. Even so, they now expect to isolate the gene itself. Once they zero in on it, they hope to learn what it does and how it causes the vast range of neurological disturbances that are typical of Huntington's disease. Eventually, their work could lead to a way to prevent or at least treat the disease and perhaps to understand other degenerative neurological disorders that also may be inherited.

Huntington's disease usually strikes in mid-life, between the ages of 35 and 45, although it can hit at any time between the ages of 2 and 80. It begins insidiously with minor symptoms such as involuntary facial twitches or clumsiness, then progresses until the entire face and body are in constant motion. Lips pout, the tongue...
darts in and out, and the eye
brows move up and down. Legs
continuously cross and un
cross, hands clench and relax,
and the gait is jerky and un
steady. The violent movements
often give way to increasing
stiffness, until the victim lies
rigid in bed. Eventually, he is
unable to talk; swallowing be
comes so difficult that death
usually occurs because of mal
nutrition, or pneumonia caused
by bits of food inadvertently in
haled while eating. The siege is
long and difficult; in a typical
case, 15 years elapse between the onset of
symptoms and death.

During that interval, the mental dam
age is equally devastating. Psychological
effects may range from depression and
schizophrenia to criminal behavior. In the
past, many people with Huntington's dis
cease were first diagnosed as mentally dis
turbed. Folk singer Woody Guthrie, prob
ably the best known American to be
struck down by the illness, for a while was
suspected of being an alcoholic because of
his unsteady walk and his slurred speech.

The origins of Huntington's disease
are lost in history, but it has been
known at least since the 1600s and
probably originated in Europe.
The disorder takes its name from a de
scription in 1872 by George Huntington, a
21-year-old American doctor who grew up
near a family of Huntington's disease vic
tims on Long Island. Huntington's pa
per—his one and only publication—cor
rectly described both the symptoms of the
disease and its inheritance. "It is con
fined," he wrote, "to certain and for
tunately very few families and has been
transmitted to them, an heirloom from
generations way back in the dim past."

That pattern of inheritance has been
the one most illuminating clue for re
searchers. The disease passes from gener
ation to generation in a way that, by Men
del's genetic rules, points to a single
dominant gene as the malevolent source.
Each child of a Huntington's disease vic
tim has a 50 per cent chance of inheriting
the gene, and everyone who has the gene
eventually develops the disease. Conse
quently, the best hope of understanding
and eventually curing the disease is to find
and analyze the gene.

This seemed like a hopeless task until
scientists developed a sophisticated bat
tery of genetic research tools and tech
iques. One key procedure involves the
use of restriction enzymes, a class of com
plex proteins that cut long strands of DNA
into shorter pieces. An enzyme
does not make its cuts just any
where; as it moves along the
DNA strand, it comes to a
short but specific sequence of
base pairs and nips through
the strand. The tiny sequences
where the enzyme cuts are
known as recognition sites.

There are probably thou
sands of the sites scattered
throughout the chromosomes.
The number and position of
the sites is inherited, just as
eye color is, and they vary
from person to person. Thus,
when a segment of a person's DNA is ex
posed to a restriction enzyme in a labo
ratory, it will be cut into fragments of vary
ing lengths that form a pattern distinctive
to that person, a pattern that he will pass
on to his children. If these segments of
DNA have a distinctive pattern and can be
found on a particular chromosome, scien
tists call them markers. Furthermore, if a
marker happens to be located close to a
gene on a chromosome, marker and gene
are likely to be inherited together.

That being the case, the researchers
reasoned, by finding a marker distinctive
to victims of Huntington's disease and lo
cating its position on a chromosome, they
would also zero in on the approximate po
sition of the Huntington's gene.

In 1979 the Hereditary Disease Fou
dation brought researchers together to dis

Left, Nancy Wexler of the Hereditary Disease Foundation with family
tree showing Huntington's victims; right, James Gusella of Massachu
setts General with an autoradiograph showing a DNA marker pattern
cuss doing just that. It was an ambitious idea, because there are hundreds of markers among the 100,000 or so human genes. Could they find one that was distinctive to victims of Huntington’s disease? The researchers decided to locate and identify as many markers as would be needed—perhaps as many as 800—to see if one was close to the Huntington’s gene. To find that marker, though, they needed to learn whether it was consistently inherited in a Huntington’s disease family. If it was, it was probably close to the Huntington’s gene. But mapping the necessary markers would take at least a decade, the investigators thought. “A lot of people were skeptical,” recalls Wexler. “But the situation was bleak, so why not try?”

A team led by James Gusella, of the Massachusetts General Hospital, began studying DNA samples taken from a large American family with a history of Huntington’s disease. The researchers had located the family through the National Radioactive Isotopes, which are easy to detect because they show up on photographic film.

After only eleven tries, Gusella hit on a promising marker, one that was present in all members of the American family stricken with Huntington’s disease. This marker has four forms, and the family members who had the disease all carried the same form. Those who were free of the disease did not carry the same form. Gusella had his first important clue.

But he needed firm evidence that these tentative findings were, in fact, pay dirt. That evidence came from Venezuela, where the NINCDS and the Hereditary Disease Foundation had investigated stories that an extraordinarily large family with Huntington’s disease was living along the shores of Lake Maracaibo. Nancy Wexler had led a team of scientists to isolated villages along the lake to chart the family tree and collect blood and skin samples.

More than a century ago, the team learned, a Venezuelan woman in the area developed the disease, although from whom she inherited it remains a mystery. She has had some 3,000 descendants, and among those living along the lake now are 100 victims of Huntington’s disease—in addition to 1,100 children who run the risk of getting it. Here were enough people to confirm a link between the gene and a marker. Wexler’s team collected samples from 570 of the Venezuelans and sent them to James Gusella.

As with the American family, Gusella found that the Venezuelans who had Huntington’s disease all carried the same form of the marker. Those free of the disease carried other forms. With the Venezuelan data, Gusella says, he was able to establish odds of 100 million to one that the marker is indeed linked to the Huntington’s disease gene. Not even Gusella, who describes himself as an irrepressible optimist, expected success so soon. Fellow geneticists had calculated that he might have to try hundreds of markers before he found one that was near the Huntington’s disease gene.

The marker pinpoints the gene somewhere within a length of several million DNA code letters. Although this stretch is large enough to include several hundred genes, it constitutes only a small portion of a single chromosome. The marker has since been mapped by Susan Naylor, of Roswell Memorial Park Institute in Buffalo, to chromosome number four, at least in the families so far tested. Gusella’s next
THE REMARKABLE PROMISE OF PROBES

DNA probes are one of the most ingenious methods yet devised to unravel the mysteries of heredity. For nearly two decades, geneticists have used these short segments of DNA to locate genes and other specific sequences of the genetic code. But now, as the discovery of the Huntington's disease marker has shown, DNA probes should become increasingly important in medical research and may soon be used to diagnose genetic disorders.

In theory probes are simple. First, scientists construct or clone a length of DNA that contains a specific genetic sequence, such as that of the Huntington's disease marker, and attach radioactive isotopes to it. Then they mix the probe with genetic material from a human cell. If the person from whom the genetic material is taken carries the sequence, the probe will find and bind to it (see illustration), radiactively illuminating its location.

So far, largely because of their radioactivity, DNA probes have been confined to the laboratories of experimental biologists, where researchers can guard against exposure to radiation. Another drawback of the radioactive probe: new batches must be prepared frequently, because the isotope's radioactivity diminishes quickly. Now, advances in probe technology are making radioactive isotopes unnecessary. Molecular biologist David Ward and his colleagues at Yale have perfected a technique employing a compound called biotin instead of isotopes. Ward incorporates biotin molecules into the structure of probes. Once the probe has been mixed with the genetic material being tested, Ward adds a protein called avidin, which carries fluorescent molecules. The fluorescent avidin binds to the biotin on the probe, signaling the presence of the target sequence—if it is there.

This simple labeling system has opened the floodgates for the manufacture of probes. Two years ago a Manhattan company, Enzo Biochem, bought the patent for the biotin system from Yale. Enzo officials think the biotin probes will be useful not only for detecting hereditary ailments but also for diagnosing infectious diseases. They foresee using probes to test body tissue or fluid for the genetic material of microbes, getting in a matter of hours information that now takes a few days. Eventually, Enzo hopes to supply laboratories with large libraries of probes. That would enable technicians to select the right one to forage out any of the hundreds of microorganisms that plague human beings.

The one sure result of locating the gene would be to simplify the test to see who has it. The test for the marker, of course, exists now, but researchers must still determine whether the Huntington's disease gene is on chromosome four in all victims before even a marker test can be reliable. Also, the marker test is still laborious, requiring geneticists to repeat each of the steps involving the probe and restriction enzyme to check each member of the family at risk. And not everyone can be tested now—only those who have large enough families to detect a linkage with the marker. The test for the gene, by contrast, will involve only a blood test of the person at risk and a probe of a DNA sample for the gene.

Even the limited testing that is possible now poses vexing questions for those who work with Huntington's victims. Who should be tested? If someone carries the marker, should he be told? And how should counselors break the news? Susan Foilstein, a psychiatrist at Johns Hopkins University, says that everyone agrees that taking the test for the gene must be entirely voluntary. But, she says, "that's not as simple as it might seem."

Insurance companies may refuse to insure people from families with the disease unless they are tested and found to be free of the gene. And once the test is no longer experimental, says Alexander Morgan Capron, a lawyer at Georgetown University, employers can legally insist that family members take it. Another sort of pressure will surely arise within the families themselves. Wives or husbands of people from Huntington's families will want to know what their future holds. Many families will want their children tested. If a man is at risk, and his wife becomes pregnant, she may demand amniocentesis to learn if their unborn child has the gene. If it does, the gene could only have come from the father. The news thus becomes a double blow: both the child and the father will become victims.

Everyone involved in Huntington's disease research agrees that potential victims should undergo extensive counseling both before they decide to take the test and after they receive the results—if the news is bad. Nearly all those at risk have already witnessed the illness and death of a parent, and often of other family members. They are under no illusions about what they face if they have the gene. An appallingly high number choose suicide: about 10 to 12 per cent of those affected kill themselves, says Connelly, and others are prepared to. "As many as thirty per cent of those at risk say, 'If it ever comes down to it, I am going to take care of myself.'"

For all the psychological risks and ethical implications, says Nancy Waxler, "the tremendous positive aspect of this is that the search for the gene is so far advanced. Everyone should feel encouraged. Whether they have the test or not, Waxler should know. Her mother died of Huntington's disease."
-CLOSING IN ON A KILLER GENE-

1. What is the treatment for Huntington's disease? How often is it fatal?

2. Huntington's cruelly kills its victims. How much time normally passes between onset of symptoms and death?

3. Is the gene for Huntington's a dominant or recessive one? What are the odds of inheriting the gene if one of your parents has the disease?