Huntington's Gene Finally Found

The long hunt for the gene defective in Huntington's disease is over, raising hopes that researchers will be able to figure out what causes the condition

Ten years ago, James Gusella thought he had in his sights one of the most sought-after prizes in medical genetics: the gene that causes Huntington's disease (HD). By analyzing the inheritance of genetic markers in families afflicted with the deadly neurological disorder, Gusella's group at Massachusetts General Hospital had determined that the gene lies near the tip of chromosome 4. Gusella and others hoped that within 5 years at most, they would isolate the gene.

So began one of the longest, most arduous, and perhaps most unusual gene hunts to date. The quick chase originally anticipated turned into a marathon pursuit as the gene, like some tricky mirage, proved agonizingly elusive. Finally, last week the long quest ended; the gene had been tracked down. And in a rare instance of "poetic justice" in science, it was Gusella's group that homed in on the quarry. But the paper announcing the discovery in the 26 March *Cell* was signed not by Gusella's team alone but by an international research consortium consisting of six groups in the United States, England, and Wales that had banded together to hunt the gene—testimony to a rare collaboration in the usually fiercely competitive world of gene-hunting.

When it came, the end of the hunt surprised even some of Gusella's collaborators. "It has been such a long roller coaster ride," says Francis Collins, who directed another part of the collaborative team at the University of Michigan. "We had steeled ourselves against thinking we would ever find it. So when Jim told me they had it, you could have scooped me off the floor."

The discovery is the first good news in a long time for HD sufferers and their families. Huntington's is a particularly cruel and insidious disease, since it typically strikes people in middle age, robbing them of their physical and mental abilities and causing death 10 to 20 years after symptoms first appear. "It's a double whammy, because by the time a person develops the disease, they often have a family, and so they must cope with their illness as well as the fate of their children," says David Housman, who led another of the consortium's teams at the Massachusetts Institute of Technology.

As a result of the gene discovery, a more direct and accurate test for HD should be possible. And ultimately, Huntington's researchers hope, the discovery will also give them a better understanding of what causes the nerve degeneration of the disease, possibly leading to a cure, even though that may take years. "Now we have a specific gene making a specific protein—and, to borrow a phrase from Clinton, we're going to focus like a laser beam on finding a cure," says Nancy Wexler of Columbia University, who bills herself as the "cheerleader" of the group that found the gene and who everyone credits with keeping the team together throughout their long, frustrating search. Moreover, defects similar to the one that apparently causes the HD gene to go awry—the elongation of a repetitive sequence of three base pairs—have recently been found in other genetic diseases such as fragile-X syndrome and myotonic dystrophy, leading some researchers to suggest that this type of genetic defect may be relatively common.

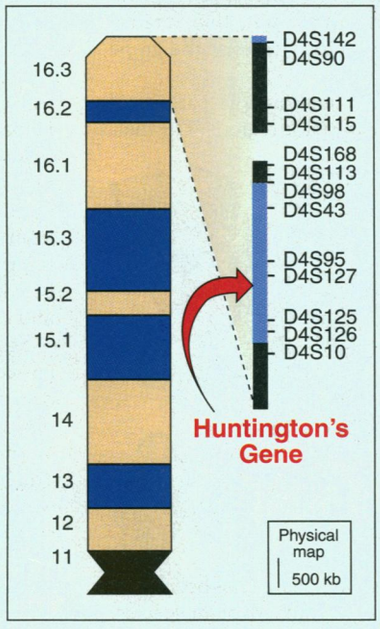
In 1984, following the Gusella group's localization of the Huntington's gene, Wexler's Hereditary Disease Foundation, a nonprofit organization her father started in 1968 after her mother's death from HD, launched the multilab effort to look for the gene. But despite the early optimism, the team, called The Huntington's Disease Collaborative Research Group, found they had their work cut out for them. Even as recently as fall of last year, few were willing to hazard a guess as to when they would find the gene (*Science*, 30 October 1992, p. 740).

During most of the 10 years of work, the group had focused much of its effort on the tip of chromosome 4, near the telomere. They were led into this region, which Gusella calls "a junkyard of repetitive DNA and no genes" by their worldwide molecular genetic studies of people with Huntington's. The chromosomes of some of those people, for example, had "recombined" so that they had apparently inherited only a small portion of chromosome 4—the part near the tip—from their affected parent. But searching for the gene in that region proved fruitless, Gusella says. Meanwhile, there was also conflicting evidence indicating that the gene might be located several million base pairs in from the telomere, but the region implicated was so long (some 2.2 million base pairs) that searching the whole segment might take years.

Instead of slogging relentlessly through this daunting stretch, however, Gusella decided to take a gamble. "We were very tired and had too many places to work on [to find the gene]," he says. He and his colleagues decided about 2½ years ago to focus on a particular region within the 2.2 million base pair region. They were led to this segment by an analysis of gene marker sequences on Huntington's chromosomes, which showed that about one-third shared a common an-

SAM OGDEN

Gene team. The team that pinned down the HD gene was led by James Gusella, shown here with Mass General colleagues *(from left to right)* Mabel Duyao, Marcy MacDonald, and Christine Ambrose. The arrow points to where on chromosome 4 they found it.



Gene Discovery Points to Better HD Test

For genetic counselors, the discovery of the Huntington's disease gene is a proverbial mixed blessing. "My first reaction was, 'This is wonderful! It's what we've been waiting for!" says Kimberly Quaid, a psychologist at Indiana University who has already tested more than 100 people for HD with a different, less precise test. "And my next was, 'Oh, hell.'" Quaid's high-low response is typical of counselors working with HD patients and their families, who know all too well the psychological trauma the disease brings with it.

Individuals who have a parent with Huntington's disease have a 50% chance of inheriting the gene and coming down with the disease, too. But because symptoms don't usually begin developing until the mid-thirties, they can spend decades worrying about whether they will get the condition, which is characterized by loss of muscle control and dementia, and ultimately leads to death. Until now, the test for the HD gene has been indirect. It involved determining whether a person suspected of having the gene had inherited certain genetic "markers" that are closely linked to the gene from an affected parent. But because this requires analyzing DNA samples from as many family members—siblings, parents, aunts, and uncles—as possible, the test is both cumbersome and expensive. And it sometimes erroneously identifies someone as having the gene when he or she doesn't.

But now that the gene mutation causing the disease has been identified, it will be possible to test directly to see if a person has inherited it. That's why Quaid and other genetic counselors are pleased by the discovery. Testing for Huntington's, she says, "will be cheaper and it will be private, which is important for some people. They won't have to tell the whole family." But such a test could have a downside as well. People who don't enlist their families' help may lose valuable support if they should turn out positive. More worrisome, Quaid says, physicians may simply prescribe the test while running, say, a cholesterol count. "Since it will be a simple blood test, it makes it easier for people to ask for, without thinking about the psychological results or personal risks, such as the effect it could have on a person's insurance, job, or relationships," says Quaid.

The counselors were less concerned about another aspect of the test—its potential ability to predict when a person who inherits the HD gene will come down with the disease. The abnormal gene carries extra copies of a trinucleotide repeat, and the greater the number of those copies the earlier the disease seems to develop. Researchers involved in the gene's discovery worry that this will further complicate the counseling of people who inherit the gene defect because it might mean that they will learn that they not only have the gene but will develop the disease early. But the counselors believe that this aspect of the test is just as likely to help patients. "For many people that is one of the hardest parts of the disease, knowing that they have it, but not knowing when it will strike," says Dorene Markel, a genetic counselor at the University of Michigan. Many patients start "symptom searching," she explains, "scrutinizing themselves at the slightest twitch or misspoken word for a sign of the disease—when, in fact, the actual illness may be decades away." Still, Markel acknowledges that the correlation could be a double-edged sword. "It can give information, but take hope away," she says, if it shows, for example, that the disease will hit at age 35 instead of 70.

Everyone agrees that there's still a need for those administering the test to counsel carefully those who wish to take it to make sure that they truly want to know whether they've inherited the HD gene and are able to deal with the consequences. "People may want to rush in and get themselves tested," says Francis Collins, a member of the large international consortium that identified the gene. "But this is not something to do frivolously and then regret."

–V.M.

cestor. The resemblance among the chromosomes was particularly strong in one 500-kilobase segment within the larger region. "I decided that the similarity was meaningful rather than merely a coincidence," Gusella says. "And that proved to be the lucky break for us." Beginning in early 1992, the Mass General group scoured that region for the HD gene, but because it was a gamble, the rest of the consortium continued to look elsewhere. By early February, Gusella knew his lab was on the right track, although, says Marcy MacDonald, who was the first to see the gene, "it was not a sudden revelation, but a slowly building recognition as we did the sequential experiments."

The researchers had pulled out and analyzed several genes, but had had trouble cloning the beginning (5′) end of one called IT15 (for "interesting transcript 15"). In January of this year, when they finally did clone and sequence that region, they found that they had extracted a large, previously unknown gene, stretching for 210 kilobases and encoding a mystery protein. At the gene's 5′ end were numerous repeats of a single trinucleotide sequence containing the bases cytosine, adenine, and guanine, or CAG, a codon that specifies the amino acid glutamine. These trinucleotide repeats proved to be the unstable part of the gene—and the site of the Huntington's mutation.

Given the HD gene's previous history, the final conclusion that this was indeed the right gene emerged in an amazingly fast 2-week push. "It took them about 10 days from the very first glimmer to when it was all done," says Collins. The group was able to move so quickly because of their previous work, as well as that of the consortium, in assembling a wealth of genetic materials from Huntington's patients and developing several technical tools for gene analysis.

During that time, they compared the gene in more than 150 HD patients with the gene in unaffected individuals. The result: People without Huntington's usually have about 11 to 24 of the CAG repeats. But the patients have anywhere from 42 to 86, the highest number found to date. In two cases, the researchers found the elongated repeat in individuals who acquired HD through a new mutation, while the gene was normal in her parents. What's more, the length of the repeat seems to correlate with the onset and severity of the disease.

Not everyone searching for the HD gene belongs to the HD collaborative group, but because the gene proved so slippery, even the group's competitors are singing its praises. "It's been a hard and fast race," says Richard Myers, who with David Cox led a rival group at the University of California, San Francisco. "And I don't want to pretend that I didn't want to win, but clearly they got it and we want to congratulate them." "We applaud their discovery," adds another contender, Michael Hayden of the University of British Columbia, who found himself in the painful position of having proposed a different candidate HD gene in *Nature* the day before the consortium published their proof-positive results in *Cell*. Yet he graciously concedes, saying, "They absolutely have it, and while it is disappointing to be wrong [about the gene's location], their discovery also gives a sense of relief. Now we can turn our attention to

other aspects of this terrible disease."

The ultimate goal, of course, is finding a cure for Huntington's. As Collins says: "That's the dream; that's what it's really all about." But finding the gene is not the same as finding the cure, and reaching that goal is certain to be every bit as eventful and frustrating as the HD gene search. Since the gene is completely unknown, researchers first have to determine what the protein produced by the normal HD gene does and how it's affected by the mutation. Presumably, Gusella says, it causes a "gain of function," whereby the gene is somehow given a new, erroneous task in the body. But finding out what that is won't be easy, given that the gene sequence provides no clues.

The researchers also want to know why the expanding, repetitious mutation affects the brain so severely, even though the gene is active in cells throughout the body. To answer such questions, Housman's group is now working on genetically engineering mice to contain the HD gene with its extra CAG repeats to see how it affects neuronal or other cell functions. "That's the high priority now," says Housman.

The researchers may have another long slog ahead of them as they look for the answers to their many questions. "We're starting with everything unknown," MacDonald says. "It's going to take some time." Researchers studying genes with expanded trinucleotide repeats in other diseases agree. "This is now the fourth disease involving this type of mutation, and I'm afraid it's a very thorny problem," says David Nelson of Baylor College of Medicine, one of the researchers who found that the defective gene in fragile-X syndrome, an inherited form of mental retardation, has extra copies of the triplet CGG. He points out that very little is known about the effects of such mutations, and the instability of the long repeats makes them technically difficult to work with.

Even though the next phase of research will be as arduous as the first, the consortium is nevertheless—and understandably—in a celebratory mood. The members are unsure if they will continue to stay together as a group. "No one has even talked about that yet," says Gusella, although he and others are sure they will all remain friends. In the meantime, the consortium plans a huge "blow out" party in May in Florida. And in Venezuela, home to a very large HD family, Wexler will have another at the Red Bull Bar, which is soon to be torn down to make way for a medical clinic and hospice for HD patients. "We're going to keep going, working and raising money to find a cure," she says. "If it turns out that our grandkids are saved because of the work we're doing, I'll be in seventh heaven."

–Virginia Morell

With reporting by Leslie Roberts.

MEETING BRIEFS

Toxicologists–and Snow–Descend on New Orleans

Neither rain nor snow nor sleet nor hail could keep 4000 toxicologists from descending on New Orleans last month (14–18 March) for the annual meeting of the Society of Toxicology. The Great Blizzard of 1993 did do some damage to the meeting program–about 300 registrants failed to show, including key speakers in sessions on dioxin science and "space toxicology." But toxicologists rebounded later in the week with a special "snow delay poster session" and a debate on regulatory approaches to dioxin. Also of topical interest: a pair of sessions on the health effects of breast implants and lead.

Lead Still a Threat Abroad

Less and less leaded gasoline and paint is making its way onto the U.S. market these days, but that doesn't mean the health threat from lead has disappeared. On the contrary, there's still plenty of it around—particularly in other countries. For that reason, some epidemiologists studying the health effects of lead are beginning to conduct their research abroad. In one session at the toxicology meeting, epidemiological studies done in the former Yugoslavia and Egypt provided new evidence of lead's health effects on children's mental performance as well as a possible link between lead and infertility.

These days, lead poisoning may be the least of people's worries in what remains of Yugoslavia, but in a study launched in 1985—before the civil wars began—a team led by Columbia University pharmacologist Joseph Graziano looked at the effects of lead on the cognitive ability of children. The group recruited pregnant women who lived either near a lead smelter in Kosovska Mitrovica, Serbia, or in a "non-lead-exposed town" 25 miles to the south. So far, the researchers have followed 334 children from birth to 4 years of age, controlling for several possible confounding variables, including the mother's age and the children's intelligence, birthweight, and gender. Unlike in past epidemiological studies, Graziano's group also controlled for hemoglobin levels, because iron-deficiency anemia has been linked to cognitive impairment.

Graziano's group found what he calls a "striking" dose-response relationship between blood lead levels and a standardized measure of cognitive function called the General Cognitive Index (GCI). The researchers saw a gradual decrease in GCI scores as the blood lead levels increased. Graziano's study adds ammunition to a camp that has argued for years that lead exposure is responsible for slight changes in GCI scores and other measures of intelligence in children. But the clinical significance remains debatable. "For a given individual it's hard to say what the consequences are. For society as a whole, if we lose 3 to 5 IQ points or more, we'll have more people who fulfill the definition of being mentally retarded who will need a special education," Graziano says.

Chronic health effects of lead poisoning, as seen in the Yugoslavian children, are hard to detect in adults. That's why epidemiologists have begun screening for such damage in adults who live in countries with heavy lead pollution. Egypt fits that bill. In Cairo, for example, the ambient air concentration of lead is 3 milligrams per cubic meter (mainly from leaded gasoline), about 1000 times higher than U.S. levels.

To test the effect of such high levels of lead exposure on Cairo men, a team of Cairo University researchers led by toxicologist Ashraf Youssef studied the semen of 30 infertile men and 25 fertile men. About half the men had extra lead exposure from their work as "traffic soldiers." The researchers found that whereas the average sperm count in fertile, nonoccupationally exposed men was about 70 million per millileter, in the most severely affected traffic cops it was 7 million per milliliter. The percentage of abnormally shaped sperm also increased in the lead-exposed group.

While the Egyptian findings are suggestive, lead-induced changes in sperm function are much harder to detect in the United States and other countries that experience less lead pollution. That's why epidemiologists are searching for biomarkers that might reveal physiological changes induced by tiny amounts of lead. One possibility is the widely used sperm chromatin structure assay. At the session, University of Washington graduate student Tim Ewers reported that in rats with blood lead levels roughly that of occupationally exposed U.S. men, the assay flagged changes in the proportion of certain sperm precursor cells produced in the testes. Washington toxicologist Elaine Faustman suggests that altered levels of precursor cells might decrease the number of sperm available for reproduction. "We have a lot more work to see if that's true," she says.