Huntington's Gene: So Near, Yet So Far

Six research groups, figuring that they can get more out of collaborating than out of competing, have banded together to look for the Huntington's gene

JIM GUSELLA WAS JUST 30 YEARS OLD when he made what was arguably the discovery of a lifetime. In 1983, using a new genetic mapping technique that was still wildly experimental, he narrowed the search for the Huntington's disease gene, which could have resided on any one of 22 chromosomes, to a stretch of chromosome 4. It was the first time anyone had used DNA markers to figure out roughly where a gene resided when they had absolutely no clues to guide them.

When Gusella and David Housman of the Massachusetts Institute of Technology started out a couple of years earlier, optimists said it would take them 10 or 15 years to find the gene this way. Pessimists predicted 50—if they could do it at all. Gusella, then at Massachusetts General Hospital in Boston, did it on practically his first experiment.

But Gusella had little time to bask in his glory before Nancy Wexler and Allan Tobin, president and scientific director, respectively, of a foundation that had given him a small grant for his gene mapping project, descended on him and said that they wanted to bring his potential competitors together to figure out how to find the gene itself.

"That is a terrible thing to say to a scientist," laughs Wexler, who is also a psychologist at Columbia University. "He had just discovered this diamond and we asked him to invite in some of the best robbers in the United States."

But Gusella agreed, and the Huntington's collaboration was born. Formally called the Hereditary Disease Foundation Huntington's Disease Collaborative Research Group, it consists of six groups that for the past 6 years or so have been sharing their ideas and materials, a bit grudgingly at first but now with increasing openness, to find the gene.

It has not been easy. After Gusella's startling discovery, luck ran out quickly, and the search has been excruciatingly slow. The group has weathered some major setbacks, like the one last year when, just as they thought they were closing in on the gene, new evidence suggested that it might reside elsewhere on chromosome 4, several million bases away (see box on p. 626). "It is definitely three steps forward, two steps back," says Francis Collins of the University of Michigan, who with the other collaborators is now settling in for the long haul.

In the highly competitive world of human genetics, where DNA probes and cell lines are guarded jealously, a collaboration this size is something of a rarity. Numerous labs worked together to find the gene for Duchenne muscular dystrophy, but Louis Kunkel of Harvard Medical School was clearly at the forefront. The collaboration between Lap-Chee Tsui of Toronto's Hospital for Sick Children and Francis Collins that finally bagged the cystic fibrosis gene last year also comes to mind, but that congenial arrangement involved just two labs—not six, with varying personalities and egos to contend with.

That is not to say the Huntington's collaboration is without problems. "It is not all love. There are tensions and paranoia," admits Wexler. "Some labs exchange more than others, some communicate better than others." There have been some notable blowups and some persistent tensions between a couple of the groups. And there are the inevitable fights over "which postdoc put what in the mail when."

But overall, it works surprisingly well. And the six groups have agreed that no matter who ultimately pulls out the gene—if it is indeed one of their group and not one of the others embarked on the same quest—there will be just one author on the paper: the collaborative group, with no individual singled out for credit.

"Huntington's shows how cooperative science can be, in the context of all being very competitive people," says Tobin.

Part of the glue that holds them together is Nancy Wexler and the Hereditary Disease Foundation that her father started in 1968 when his wife was diagnosed with Huntington's—a devastating, uniformly fatal disease characterized by progressive loss of motor control, personality changes, depression, and dementia. It is an autosomal dominant disorder; just one gene from one parent will bring on the disease. And that means Nancy Wexler herself has a 50% chance of developing Huntington's, which usually hits people in their 40s. She is 44.

"Knowing her status, you can't look her in the eye and say 'I can't work with so and so,' " says Collins. He and others say it is largely Wexler's drive and enthusiasm that persuaded six lab chiefs to work on this rare disease, which affects just one in 10,000 people, and keep at it year after year. Collins credits her with "an amazing sixth sense of who needs encouragement."

And part of what binds them is that the work is so unimaginably hard. The long-sought gene lurks out at the end of chromosome 4 near the telomere, a messy region full of DNA repeats and strange things, like a defunct retrovirus, where no rules hold. John Wasmuth of the University of California at Irvine, another collaborator, calls it the "twilight zone of gene cloning."

Roy Gumpel

Beaching it. *The Huntington's collaborators in the Florida Keys where they meet each April for sun and science. Nancy Wexler (left) is part of the glue that holds the group together through both progress and disappointment.*

A year ago the group thought the gene was practically in hand. Hans Lehrach's lab at the Imperial Cancer Research Fund (ICRF) in London had finally cloned the end of the chromosome, where almost all the evidence said the gene should be. But things unraveled 9 months or so ago when several groups came up with contradictory evidence that placed the gene not at the end, where most have focused their search, but several million bases in toward the middle. The bulk of the evidence still places the gene at the tip, but the new data are beginning to look all-too convincing, says Gusella.

"It is driving everyone crazy. It is the slipperiest gene I have ever encountered," says Wexler.

Last January the group predicted they would have the gene within a year. Now all bets are off. In the worst of all worlds, it could take another 3 or 4 years, says Collins. "I hate to think how long it would take without the collaborative group."

The collaboration actually had its origins in 1968, when Leonore Sabin Wexler was first diagnosed with Huntington's. Her husband, Milton Wexler, assembled some of the best biologists around, like biochemist William Dreyer and geneticist Seymour Benzer, both at the California Institute of Technology, and asked them what his new foundation should do to cure the disease. They advised against spending his money on bricks and mortar and told him instead to find the brightest young scientists in the country and interest them in the disease.

Milton Wexler enlisted Ronald Konopka, then a graduate student in Benzer's lab, to do just that—to travel around the country, talking to people in various labs to find out what they were doing. He found a dozen or so postdocs and invited them to the first of a series of free-wheeling, interdisciplinary workshops the foundation still holds several times each year. Tobin, then a molecular biologist at Harvard, went to one in 1972 and got hooked. Now at the University of California at Los Angeles, Tobin became scientific director of the foundation in 1978.

Those were the early days of recombinant DNA, when researchers were first using this technique to isolate genes, and Tobin insisted to the skeptical neurobiologists on the foundation's board that it could help them track down the Huntington's gene. He set out to recruit molecular biologists to work on the problem.

The first person he turned to was David Housman of MIT, with whom he had shared a babysitter in Boston. Housman had already begun to think about tackling Huntington's after a chance encounter with Joseph Martin, chief of neurology at Massachusetts General Hospital, who was setting up a Huntington's center there.

Watermelon kids. *Some of the youngest members of the huge Venezuelan family that has donated its DNA to the quest for the Huntington's gene.*

Housman and Tobin organized a workshop for the Hereditary Disease Foundation at the National Institutes of Health in October 1979 to look at how molecular genetics could help. "It was complete pandemonium, total chaos," recalls Wexler. "Everyone was yelling and screaming. David Botstein (then at the Massachusetts Institute of Technology) would go to the board and scribble furiously." Then others would jump up.

The issue was whether a new type of genetic mapping, could really be used to find genes like the Huntington's gene or the cystic fibrosis gene, when there were no clues to go on—no information about its general location, its structure, or its biochemical role in the cell. The whole strategy hinged on a new type of DNA marker that had just been detected—a restriction fragment length polymorphism, or RFLP. Pronounced "riflip," these are simply places along the chromosome where the DNA sequence varies among individuals. Botstein, Ray White, then at the University of Massachusetts, and others realized that these markers, if they were as abundant as everyone believed they were, could be used as landmarks in the search for genes.

The idea was to analyze the DNA of families who carry a gene for an inherited disease to see if the disease trait is inherited along with any particular RFLP marker. If the two consistently show up together, the disease gene is then "linked" to the marker and must be located close by on the same chromosome.

No one actually believed that anyone would be crazy enough to look for an obscure disease gene with an untried approach, says Wexler. But Housman was. He went back to try it with his graduate student Gusella, who soon took over responsibility for the project, first at MIT, and then at Mass General, where Gusella joined Martin's new Huntington's Disease Center Without Walls.

Meanwhile, Wexler began working with a family that would prove crucial in localizing the Huntington's gene. At the workshop she asked Housman whether the size of the family used in these linkage studies mattered. The bigger the better, he responded. Wexler knew of a big one and said she would look into it—a huge extended family living in fishing villages around Lake Maracaibo, Venezuela.

She then began an effort that continues to this day to trace the inheritance of Huntington's in this family and to collect blood samples to supply DNA for the linkage studies. This family is now thought to represent the largest concentration of Huntington's disease in the world, with at least 144 living affected members and more than 1000 at risk.

It took a couple of years to scale up—for Gusella to find the RFLP markers and Wexler to collect blood samples. Meanwhile, Michael Conneally, a geneticist at Indiana University, had identified a large multigenerational family in the Midwest with the disease and was also supplying blood samples to Gusella.

Gusella began testing markers on DNA from the midwestern family in the summer of 1982. The third one he tried scored a hit, though the signal was weak. Linkage is measured in something called a lod score, and this marker scored only 1.7, which is

suggestive but far from proof. A lod score of 3 is considered proof of linkage. He then tried the marker with DNA from the Venezuelan pedigree. The lod score jumped to 6, which meant 1 million to one odds in favor of linkage.

"It blew the socks off everyone," says Wexler. Even Botstein, one of the chief architects of RFLP mapping, says it was Gusella's discovery that made the entire procedure credible.

Gusella didn't know it yet, but he had landed about 4 million bases away from the gene—not bad, considering there are 3 billion bases in the human genome. But it would take Gusella another year or so to figure out where on chromosome 4 the marker, known as G8, was located.

The question, then, was how to get from the marker to the gene itself, which was totally uncharted territory in those days. That is when Wexler and Tobin asked Gusella to help them organize a January 1984 workshop to bring together molecular biologists and cell geneticists who had techniques that might help to find the gene. No one knew what to expect going into the workshop, but by the end of the day a collaboration of sorts had emerged spontaneously, with various people offering to tackle discrete parts of the problem.

To Gusella the decision was easy. True, he might lose out on the glory, but he had an

Three Steps Forward, Two Steps Back

The first thing that got them worried was a strange chromosome in just one patient, a member of the huge Venezuelan family that has been so helpful in narrowing the search for the Huntington's gene.

Before that patient cropped up, almost all the evidence had pushed the Huntington's gene farther and farther out toward the tip of chromosome 4, near the telomere. Most recently, for example, James Gusella of Massachusetts General Hospital had found three patients who appeared to have the gene in the last 100,000 bases of the chromosome.

Gusella and his colleagues had originally localized the Huntington's gene to chromosome 4 by showing that it is inherited along with certain DNA sequences called markers that they mapped to that chromosome. But these three patients had chromosomes that looked completely normal, without the characteristic Huntington's markers, even though they clearly had the disease. The most likely explanation for them, Gusella says, is that the defective gene is located at the extreme tip—in the last 100,000 bases—which investigators can't "see" because they don't have any genetic markers in that region.

Gusella and the other members of the Huntington's collaboration (also see story on p. 624) were understandably elated in January 1989 when Gillian Bates, a postdoc in Hans Lehrach's lab at the Imperial Cancer Research Fund in London, finally cloned the end of the chromosome. With any luck, that piece of DNA contained the gene itself. Some of the group predicted that they would find it within a few months, or at the outside, a year.

But the new patient "threw a monkey wrench" in the effort, says Gusella. He has the distinctive Huntington's chromosome markers, but only to a point about halfway up the short arm of chromosome 4, to a place slightly above the first marker Gusella found in 1983. Then the chromosome switches to the normal, or wild, type clear to the end. Since the patient has Huntington's, it would seem the gene cannot be at the end but must be located several million bases in.

Gusella and the other collaborators first assumed that there had to be a mistake—that the patient was misdiagnosed and really didn't have Huntington's. Or perhaps there had been a lab screwup, and the wrong sample ended up in the vial. The patient was rediagnosed by a neurologist. Blood was drawn again. DNA samples were prepared again. Everything was quadruple checked, says Gusella. Not only did the data hold up, but Gusella found a second patient with the same unexpected pattern of markers. And another group outside the collaboration found a third. That left them with what seems to be solid—and contradictory—data. One set suggests the Huntington's gene is at the tip and the other points to a more internal location for it, perhaps several million bases in.

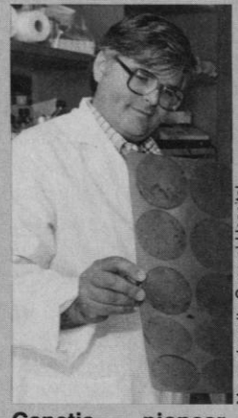
The only explanation, other than the unlikely possibility of two separate mutations, is that some rare genetic event has occurred in one set of the patients—perhaps a double recombination or a gene conversion, which makes the data misleading.

But which model is wrong? At first most of the group tended to believe the data pointing to the end location. But then Peter Harper at the University of Cardiff and Michael Hayden at the University of British Columbia both found evidence of linkage disequilibrium, which usually means you are very, very close to the gene. And it, too, indicated that the gene was located several million bases in from the tip.

But, as has been the norm throughout this long quest, these new data were also strange and inconclusive. Instead of finding one spot on the chromosome in strong linkage disequilibrium, they have found two places in weak equilibrium—and they are more than 1 million bases apart.

To Francis Collins of the University of Michigan, "the handwriting on the wall is becoming clear," and it points to the internal location. Gusella, too, is betting on the middle location, partly because the telomere region is so strange that "it's hard to imagine one would bury a coding sequence out there." And he says, "I don't want to work in the mess at the end. It is not fun."

At this stage, the investigators are hedging their bets and looking for the gene in both locations. In the worst of all worlds, they will have to plow through the entire 1 million bases in the more internal chromosome region looking for active genes—and there could be 30 or 40 of them—and then evaluate each one to see if it is the Huntington's gene.



Massachusetts General Hospital

Genetic pioneer. *James Gusella localized the Huntington's gene.*

Identifying the gene will be tricky, says Gusella, because "you don't have a clue about what you are looking for." No one knows what the gene does and how it selectively kills certain cells in the brain, bringing on the characteristic jerky movements of Huntington's and finally dementia and death. Nor do they know why the gene, which lurks on the chromosome since birth, only wreaks its damage in midlife, or in which tissues it is active. One would assume in brain tissue, but that is not necessarily the case. It could produce a toxin somewhere else that is then transported to the brain.

Says Gusella: "If the gene is in that 1-million-base-pair region, and we happen to be unlucky and have to look through 990,000 before we hit the right 10,000, then it could take a very long time."

■ L.R.

inkling of how long it would take if he went it alone—and he wanted to find the gene fast. Gusella also knew that although he had the lead now, he could easily be swamped by a bigger lab, or a lab with a new technique.

"People collaborate for all kinds of motivations," says Conneally, who with Glen Evans of the Salk Institute and Bob Horvitz of MIT, advises the collaboration. "We would like to think it is purely altruistic but it is not always. It is a calculated risk you are taking. You give up some autonomy—and if you find the gene, you have to share it; you don't get all the laurels. But on the flip side, if you collaborate and someone else finds it, you get a share of the brownie points."

Within about a year, the collaboration became official. The foundation agreed to give each of the investigators $30,000 a year to support a postdoc and augment their other grants. (They've since raised the amount.) In exchange, the investigators agreed to distribute unpublished information and materials freely within the group and not to send them to outside labs without permission.

A few groups have come and gone over the years, but a steady core remains: Housman of MIT; Gusella of Mass General; Wasmuth of Irvine; Collins of Michigan; Charles Cantor and Cassandra Smith, then at Columbia and now at Lawrence Berkeley Laboratory and Berkeley; and Lehrach and Anna Marie Frischauf, then at the European Molecular Biology Laboratory and now both at ICRF.

The group in turn is in a friendly competition with the other major labs looking for the Huntington's gene: Rick Myers and David Cox of the University of California at San Francisco, Peter Harper of the University of Cardiff in Wales, and Michael Hayden of the University of British Columbia.

By mid-1986, the collaborators had found that the G8 marker was located near the end of the short arm of chromosome 4—and that the gene itself was in a region of about 5 to 10 million bases on either side of it.

But which side? They needed more markers from the region to find out, but getting them was slow going. The group watched in envy as their colleagues tracking down the cystic fibrosis gene first found linkage and then almost immediately found flanking markers that narrowed the search to a region about one and a half million bases long. No such luck with Huntington's. Try as they might, the collaborators could not find another marker out near the tip, much less a flanking marker to bound the area.

Finally, a marker donated from a researcher outside the collaboration turned out to be close. And it told them that the gene was above the original marker, out toward the tip. At least they knew which way to look. Then Conrad Gilliam in Gusella's lab found a second marker in the region. It looked like it was right on top of the gene.

That is when the collaboration really got going, says Gusella, as they all brought their diverse techniques to bear on the problem. And that is when things got tense as well. Although everyone had agreed to collaborate, until then they hadn't realized just what they were in for, says Wexler. Tempers flared at a meeting in Boston in 1986, with charges of withheld data, unreturned phone calls, and probes lost in the mail.

The ostensible problem was that they were all planning to do essentially the same experiment. But the real issue, says Wexler, was simply, "How much can I trust you? Were others being honest or were they going off to be the Lone Ranger?" At that time everyone thought it would be easy to find the gene, she says, and part of the tension was the fear that someone in the group would find it and clone it before the others even heard about it.

But that wasn't the case, and the difficulty in tracking down the gene has helped pull the group together. "People found they needed each other," says Wexler. "You get tired of hitting your head against a brick wall and don't mind a little help," agrees Lehrach.

"You get tired of hitting your head against a brick wall and don't mind a little help."

The closer they got to the gene, the more it eluded them. Additional family studies showed that the new marker was not right on top of the gene after all. And they were increasingly confounded by the weirdness of the region out near the telomere, which stymied efforts to develop a physical map that could help locate the gene. The genetics were getting more perplexing as well. Almost every time they found a new marker even closer to the tip of the chromosome, the genetic analysis indicated that the gene was located still further out. They were beginning to think they would never reach the end of the chromosome.

So when Gillian Bates in Lehrach's lab finally cloned the end of chromosome 4 last January, the group was elated. But then came the setback of last summer: accumulating evidence suggested that the gene was not at the tip but perhaps several million bases in toward the middle. Now the investigators are stuck with contradictory data, pointing to two different locations.

The group is just back from its January workshop in Los Angeles—and a party at Blake Edwards and Julie Andrews' house—where they tried to sort out the confounding data. They decided to divvy up the search; some labs will continue scouring the telomere region looking for the gene, while others step up the hunt several million bases away. All agree that it could be a long slog.

The January get-together, held at a low point in the quest for the Huntington's gene, demonstrates another part of the glue that holds the collaboration together: a congenial blend of science and socializing designed to keep momentum—and spirits—up. They meet three or four times a year to compare notes, chart strategy, or commiserate, as the case may be. The January meetings are always held in Los Angeles, where the foundation throws its most lavish parties. Blake Edwards and Julie Andrews are regulars, as are Carol Burnett, Jack Lemon, Jennifer Jones, and other stars.

And in April it's the Florida Keys, where Dennis Shea, one of the foundation's supporters, has converted his guest house to a conference center for the group. Much of the work there is done in bathing suits.

The postdocs go along, too, because it is largely their ability to work together that keeps materials flowing among labs. But it's a rare postdoc willing to work in the collaboration, says Gusella. "Postdocs are out there to be famous, to come up with important papers to make their career. There is very little in the formal training process that teaches grad students and postdocs how to work together." Those who do thrive, he says, are generally "very confident and are not completely driven by ego. They are people who like science"—people like Marcy MacDonald in his lab, Michael Altherr in Wasmuth's lab, and Bates in Lehrach's lab.

Perhaps the greatest worry all along has been whether the collaboration would hold up once they got close to the gene, or whether someone would make a solo bid for glory. The agreement on the final publication—if indeed there is one—eased those tensions enormously, says Wexler, who calls publications the bane of any collaboration. "We finally agreed that the only way to guarantee that cooperation will continue is if you are not going to lose anything," says Gusella. "And the easiest way to do that was to essentially eliminate authorship."

And they all echo Collins' sentiment: "It would be nearly impossible to sort out people's roles and contributions anyway, and it is not worth trying. Everyone has contributed their own blood."

■ **LESLIE ROBERTS**