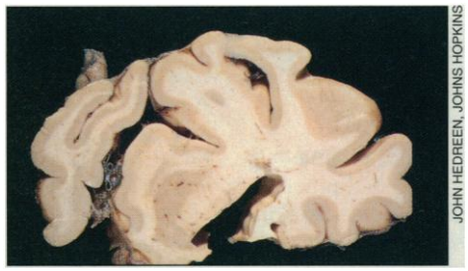
The Huntington's Gene Quest Goes On

Confronted with perplexing data in an unusually difficult region of chromosome 4, investigators are nonetheless closing in on the Huntington's gene

Nancy Wexler calls it "a long day's journey into night"—the seemingly endless slog to find the genetic defect that gives rise to Huntington's disease. Although the Huntington's gene was the first to be mapped with then new DNA markers in 1983, a feat of technical prowess and uncanny good luck, that luck soon vanished. Indeed, since that initial success, by Jim Gusella at Massachusetts General Hospital, the search for the gene has been a nightmare of false leads, confounding data, and backbreaking work. For a decade Huntington's researchers have watched as their colleagues tracked down the genes involved in cystic fibrosis, muscular dystrophy, fragile X syndrome, and myotonic dystrophy, to name a few. But the Huntington's gene has remained elusive.

Now, 2 years after the quest reached its nadir—Huntington's investigators realized in 1990 that the data had been pointing to the wrong place altogether—investigators, including the multilab collaborative group started by Wexler's Hereditary Disease Foundation, are upbeat. They are reasonably sure they are looking in the right region, near the end of the short arm of chromosome 4. What's more, they have cloned all the DNA there and are actually pulling out genes—one of which could be the Huntington's gene. Not that they're assured of success within the year, however. The region may house 100 genes and, given their luck to date, the researchers fully expect they may end up sequencing and analyzing every single one, or worse yet, the entire 2 million-plus base pair stretch, before finding the culprit. Perhaps only Huntington's investigators, who have weathered so many setbacks along the way, could consider themselves in the home stretch.

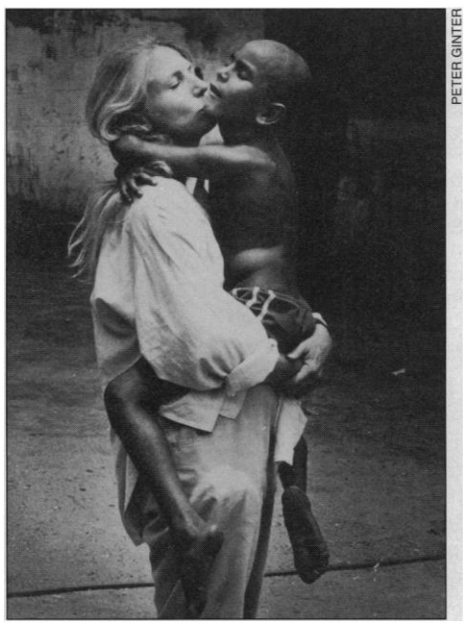
What has turned the Huntington's quest into such a punishing marathon? For one, the various teams seeking the gene have had no biochemical clues about what the gene does or how it kicks in, usually in midlife, leading to massive cell death in the brain, uncontrollable movements, intellectual and emotional impairment, and ultimately death. "It is a completely cataclysmic disease. It is totally unremitting," says Wexler, a psychologist at Columbia University whose mother died of Huntington's and who is thus at risk herself. True, other successful gene hunters started out similarly ignorant of their gene's exact function. But in most other cases—in muscular dystrophy and neurofibromatosis, to name just two—the investigators were blessed with physical clues to guide them when the biochemical ones were lacking—namely a translocation or gross rearrangement of the chromosome that clearly delineated the suspect region. But not so the Huntington's researchers. "It's what we dream of," says Wexler wistfully.



JOHN HEDREEN, JOHNS HOPKINS

Huntington's damage. A section of the brain through the striatum shows severe atrophy.

This has left the Huntington's investigators with just "recombination events" to guide them, which can be more confusing than definitive. Indeed, these events, which occur when two homologous chromosomes exchange pieces, point to two entirely different locations for the Huntington's gene. To add insult to injury, both are out near the tip or telomere of chromosome 4—a fantastically difficult region to work in. This telomere, in particular, is so full of repetitive DNA and bizarre behavior that Wexler likens it to a "genetic San Andreas fault." One location in this wasteland would seem to be wrong, but no one has found a way to figure out which it is—much less why the conflicting data won't go away.



PETER GINTER

Persistent pursuit. Nancy Wexler, here with an unusually young Huntington's patient, has chased the gene for 10 years.

That quest alone has consumed much of the past 5 years, not just for the foundation's collaborative group—now composed of Gusella, Francis Collins of the University of Michigan, Peter Harper and Duncan Shaw of the University of Wales, David Housman of the Massachusetts Institute of Technology, Hans Lehrach of the Imperial Cancer Research Fund in London, and John Wasmuth at the University of California, Irvine—but also for Richard Myers and David Cox at the University of California, San Francisco, and Michael Hayden of the University of British Columbia, who are tackling the gene hunt independently.

Confounding data and quirky genetics

This beleaguered band of investigators has had some help from the worldwide genetics community, who pitched in almost as soon as Gusella announced in 1983 that the gene was located within the top 6 million bases of chromosome 4. Six million bases was far too huge an expanse in which to hunt for a gene, so investigators worldwide began looking for "recombinants," people with Huntington's disease whose chromosomes have recombined in that region, to narrow the search.

The strategy is straightforward: With enough markers along the chromosome, which serve as lamp-posts to light the way, investigators can test offspring in Huntington's families to see which section of chromosome 4 they inherited from which parent. If because of a recombination event only part of the Huntington's chromosome was passed from an affected parent to the child, who also has the disease, then the mutation must obviously reside in that chunk. Before long, investigators identified about a dozen of these individuals.

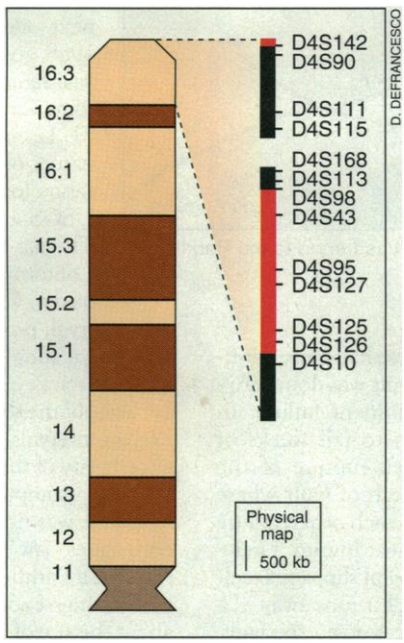
But from the outset, the data were perplexing. The most convincing evidence kept pushing the gene farther and farther out toward the tip of the chromosome. For example, Gusella, Marcy MacDonald in his lab, and others found individuals who clearly had Huntington's but appeared to carry the normal chromosome, without any of the marker patterns associated with the disease. Since

the investigations had no markers that would allow them to "see" the very tip of the chromosome, they assumed that a recombination must have occurred at the tip and that the defective gene must be there as well. What seemed to clinch the case were genetic data from several families that placed the gene in the last 100 kilobases of the chromosome.

True, there was conflicting evidence. Investigators had turned up a few other Huntington's patients who had a section of the characteristic Huntington's chromosome in a region just above Gusella's first linked marker, D4S10. But above that, toward the tip, the chromosome appeared to be normal. At face value, those data would place the gene at a more internal location, several million bases in. To reconcile the data, the investigators reasoned that those individuals must have a second recombination at the very tip that they couldn't detect because of the lack of markers there. If the researchers could only see the tip, they postulated, they would find the chromosome switched back to the Huntington's version.

These findings kicked off a furious quest to find markers at the tip and to clone the end itself. But when Gillian Bates in Lehrach's London lab, and Myers and Cox in San Francisco, finally cloned the last several hundred kilobases of the chromosome in 1990 it didn't look right. There was no sign of the putative recombination event. What's more, the DNA was chock full of repetitive sequences, which were unlikely to house any genes. "The whole paradigm collapsed," says Collins.

The new data meant that the other recombinant events, which targeted the much larger internal region, had actually been pointing in the right direction. What's worse, the investigators would have to wade through not just 100 kilobases in search of the gene, but more than 2 million. Now the challenge is to explain what Wexler calls the "ghost" recombinants that pointed them to the tip in the first place. They suspect a rare genetic event, like a gene conversion, is at play. In a gene conversion, just a tiny piece of DNA "jumps" from one chromosome to another. So in these ghost recombinants, they postulate, the defective Huntington's gene somehow moved into an otherwise normal chromosome but they can't "see" it because it is so small. To Wexler, the conflicting data suggest "something unusual genetically" in the region, which appears very prone to recombination. "Maybe the area is really volatile, with tiny volcanoes erupting all over the place that we can't see." Whatever the explanation, the investigators have decided to ignore the telomeric region while they scour the huge internal region for genes.



Where's the gene? The two candidate regions *(red)* for the Huntington's gene are at the tip of chromosome 4.

Needle in a haystack

The investigators have spent much of the past 2 years laying the groundwork for an all-out assault on the gene. Earlier this year, Bates in Lehrach's lab and Jian Zuo in Myers' lab successfully cloned the entire 2.2 million base pair region. "It is a significant psychological boost," says MacDonald in Gusella's lab. "Now we physically have the region in little test tubes and can apply old and new methods to it. It is just a matter of looking at it all."

At the same time, MacDonald, Peter Harper, and Michael Hayden, and others have been trying to narrow the candidate region, looking for more recombinant individuals—so far to no avail—and performing another type of analysis to detect what is called "linkage disequilibrium," a complex genetic phenomenon that suggests the researcher is very close to a gene. But again, in keeping with the peculiarity of the region, the results are confounding. Instead of a clear signal pointing to one location, they found weak signals in several places over a 500-kilobase stretch. Still, to investigators starved for a break of any kind, this is better than nothing. Says Collins: "At least it skews the odds toward the notion that the HD gene is going to be in that 500-kilobase interval. So all of us, while banging away on the whole 2.2 megabases, are particularly intensely banging away at that 500 kilobase interval."

So far, the collaborative group has pulled out perhaps 20 genes from the entire region, while Cox and Myers have found seven, and other groups have found still more. The trick, though, will be determining which is the long-sought Huntington's gene. Each new gene must be evaluated, but what should the researchers look for? Although the Huntington's gene wreaks its havoc in the brain, the researchers can't even assume it is primarily expressed there, says Wexler, who notes that the gene causing phenylketonuria, which has a profound effect on the brain, is expressed in many tissues. Nor do they know if the Huntington's gene kills directly, by producing a cytotoxin in abnormal quantities or in the wrong place, or indirectly, by rendering brain cells vulnerable to other toxins.

Some clues are emerging from the new neuropathology collaboration that Wexler's Hereditary Disease Foundation started last year. They have turned up some tantalizing hints as to which cells in the striatum are the first to die (the striosomes it seems, not the matrix) and how the Huntington's gene might act (perhaps by impairing mitochondrial metabolism). If borne out, these leads could help in evaluating which is the Huntington's gene, but in the interim the geneticists have no alternative but to treat every gene as a serious contender.

Whatever the gene does, Collins expects the mutation to be very subtle and thus very difficult to find. He puts his money on a point mutation. "We will look for a single amino acid change that causes a protein that normally does X to now do Y." Even harder to detect, he says, would be a mutation in a regulatory region that causes a gene that wouldn't otherwise be active in the brain to be expressed there, or one that increases the stability of its messenger RNA so that it builds up in the brain rather than degrading.

Ultimately, says Collins, "you can't reject any of these genes in that interval until you have sequenced the whole blooming thing," comparing both the normal and disease versions to look for mutations. "If you find nothing you still have to worry that you missed it," he says, because you didn't sequence enough of its regulatory sequences. "You can create a whole list of nightmares for this disease and keep yourself up very late every night if you let your imagination wander a little too far."

To avert just such a scenario, other groups, like Housman's at MIT, are looking for mutations in noncoding as well as coding regions. In the end, if the gene does not pop out anytime soon, the Huntington's community may end up sequencing the entire 2.2 million base stretch—a daunting task, since, as Myers says, "No one is good yet at sequencing 2.2 megabases of genomic DNA."

Wexler is hoping to persuade one of the large-scale sequencing labs to take on the task. If none will, then the collaborative group, which has already begun sequencing on a small scale, is game to try. "It is an amazing group of people," says Wexler. "They are still very energetic. Come hell or high water, they have to find the gene." And nightmare scenarios aside, everyone is convinced that they will find it. Collins is the only one foolhardy enough to hazard a time estimate, which he puts at 2 years. But, he adds, it could be tomorrow. As Wexler says, "All we need is luck."

–Leslie Roberts