NEUROSCIENCE MEETING

HUMAN DISEASE GENES ELUDE IDENTIFICATION

TORONTO, Ont.—“On Chorea,” the only scientific treatise by Long Island, NY physician George Huntington, is over a century old. Yet, despite decades of concentrated effort by scores of research scientists, the gene that causes Huntington’s disease remains unidentified. So, too, for most inherited nervous system disorders—except retinoblastoma and Duchenne’s muscular dystrophy. Classical genetic analyses have narrowed the possibilities in many cases, and modern molecular biological and recombinant DNA techniques often have brought researchers within tantalizing distance of the actual disease-causing gene. But the search continues.

What makes this problem so tough? For neurofibromatosis (as for other diseases), “A couple of decades of research at the cell culture level have not yielded any terribly promising clues as to what the gene might code for,” explains Francis S. Collins (University of Michigan Medical School, Ann Arbor). “That means the only way you’re going to clone this gene is indirectly, using knowledge of its map position.” Speaking at the 18th annual meeting of the Society for Neuroscience here in November, Collins described just how formidable this job can be. “It’s a problem of scale. You’re starting at the outset with the entire human genome as the gene’s possible location. Out of six billion base pairs, you’re looking for one gene, and to make sure it’s the right one, you’re looking for a mutation. And the mutation might be as small as a single base pair (as it is in sickle cell anemia).” Even after the approximate chromosomal location is known, there’s still a lot of DNA to deal with; an average gene is about 10,000 base pairs. “At that level,” explains Collins, “you might be able to pick up some gross rearrangements...but if you’re after a single base pair change...your technology has to be pretty clever.”

The saga of the hunt for the neurofibromatosis (NF1) gene illustrates the challenge involved. An autosomal dominant disorder generally known as elephant man’s disease, NF1 is surprisingly common. According to Collins, the incidence is one in 2,500 for all races that have been carefully studied. And the spontaneous mutation rate is very high, about 10^{-4} per allele per generation (100-fold the normal rate). “About half the cases seen in NF clinics represent new mutations. Neither parent is affected,

nor are members of previous generations,” he says. “What is puzzling is the variation in the disease’s severity, both within and between families...In a given family, you might find individuals who are so mildly affected that they are unaware they have the disease, and others who are absolutely devastated...This implies that there are factors in addition to the germ line mutation that contribute to the disease.”

Characteristically, NF1 sufferers have skeletal deformities, multiple benign neurofibromas, and nodules on the eye’s iris. The more severe complications affect about one-third of the NF1 population. Optic gliomas develop in 15–20 percent of affected individuals and, in 3–5 percent, the

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neurofibromas develop into neurofibrosarcomas. “You might say this gene is in some way involved with growth regulation, especially of the peripheral nervous system,” he concludes.

Armed only with those few paltry clues, Collins, his many collaborators, and additional research groups started to track down the NF1 gene.

The first step was to put the gene on a chromosome. This is usually done by linkage analysis of affected families—not a problem with a disease as common as NF1. The gene even segregates to Mendelian perfection: it is inherited by 50 percent of the offspring of affected individuals. “A number of groups who had been analyzing NF1 pedigrees pooled their data in early 1987,” says Collins. “At the time it looked as if about 80 percent of the genome had been excluded. This allowed people to focus their efforts. Very shortly after that two groups presented evidence that linked the NF1 gene to chromosome 17.” Since then, researchers have identified markers ever closer to the gene—for one of these, the odds in favor of it being linked to NF1 are 10^{26} to one. Another marker is so close that it pushes the limits of linkage analysis, even though it’s still about 600,000 base pairs down the road.

To get closer to the NF1 gene,

researchers have inserted small chromosomal segments—containing a translocation breakpoint near the NF1 gene—into rodent cells to create somatic cell hybrids. With enough different hybrids, DNA interval maps have emerged that match the linkage order precisely, explains Collins. One of these breakpoints, he adds, probably interrupts the NF1 gene itself.

Somatic cell hybrids may be useful, says Collins, “but you still need a physical map to indicate how many base pairs you’re dealing with.” And to accomplish that, pulse field gel electrophoresis has proven the most powerful tool—because it separates very large pieces of DNA. “By blotting a gel and constructing a physical map, you can take all your genetic markers and see whether they connect to each other...If two probes both hybridize to one fragment that is about 950 kilobases, then you know they are no more than 950 kilobases apart.”

But the physical map isn’t enough, either. To really home in on the gene, it’s necessary to start cloning the DNA that surrounds it. And for this, Collins explains, “We’ve relied on chromosome hopping, which allows you to travel from your probe to the disease gene rapidly. Basically, you don’t clone the DNA in between...you simply hop over it and then clone something at the other end...It’s not only quick, but an advantage when something in that interval is difficult to clone.” To date, this hopping technique has generated 71 new pieces of DNA that are in the interval where the gene ought to be, says Collins. Perhaps one of them contains the NF1 gene.

The gene for Huntington’s disease has proved just as intractable. James F. Gusella (Harvard Medical School, Boston, MA) explains that, even after close to 100 years of research on the disease, “We knew it was a genetic disorder characterized by programmed, localized nerve cell death, and that was about it.”

It didn’t take Gusella and his collaborators long to identify a cosegregating marker—located on chromosome 4—whose odds of being located somewhere near the disease gene were greater than 100 million to one. That was in 1983. “In terms of scale, we had moved down from six billion bases to maybe 200 million. We have spent all the time since then trying to get it down to maybe one base, and we are not nearly there.”

—Jennifer Van Brunt