The Race for the Cystic Fibrosis Gene

When the stakes are high, emotions sometimes get the better of good intentions, and the rules get bent, if not broken

ABOUT a year ago Robert Williamson thought he had it. After years of struggle and false leads, he announced that he had found a "candidate" gene for cystic fibrosis—a devastating illness that afflicts 30,000 children and young adults in the United States alone. With that announcement, Williamson became the apparent victor, among a handful of research groups, including one biotechnology company, in a particularly bitter 6-year quest for the gene.

But by the end of the summer, when no new information was forthcoming from Williamson's lab at St. Mary's Hospital in London, his competitors, some of whom had stopped their own hunt for the gene, began to get suspicious. And when Williamson admitted in October that he had been wrong, that his "candidate" was not the long-sought gene, the envy many of his competitors felt turned to anger.

Williamson's friends say his enthusiasm got in the way—that he acted too hastily, and with too much confidence, given the evidence at hand, raising false hopes for the patients and their families in a field where there had been more disappointments than successes. Others accuse him of showmanship and shoddy science, of holding back data, and actually slowing progress toward the gene.

Even within the highly competitive field of human genetics, the search for the cystic fibrosis gene stands out for the intense nature of the rivalry. The quest has involved some elegant science, admirable collaboration, and great dedication among researchers who have devoted years to finding the gene. There has also been some reportedly atrocious behavior. "People have done things that are unthinkable in academic science," muses one observer. "This is not your average ego-driven science. This is nasty."

Rumors abound, many of them clearly unfounded, as do legitimate complaints about data withheld and probes never sent. Much of the rivalry is surely due to personalities. "Big diseases attract big egos," quips another geneticist on the outside. But it also arises because the stakes, both societal and personal, are so high.

Cystic fibrosis is the most common fatal inherited disease in the Caucasian population, affecting 1 in 2000 live births, and the disease is devastating for the children and family alike. It is characterized by a thick, viscous mucus that clogs the airways in the lungs and harbors bacteria. Most children are diagnosed in infancy, when they are plagued by repeated lung infections that will persist throughout their lives. The disease also leads to pancreatic failure and, in turn, to malnutrition.

This is the first of a two-part article chronicling both the research and the sometimes intense personal rivalries in the quest for the cystic fibrosis gene.

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With high doses of antibiotics to ward off infection, enzyme supplements to counter malnutrition, and physical therapy to loosen the mucus in the lungs, life expectancy has been extended dramatically, and some patients now live into their 20s or even 30s. But the disease is inevitably fatal.

"It would be one of the most gratifying diseases to solve," says Arthur Beaudet of Baylor College of Medicine, echoing the sentiments of many involved in this quest. Finding the gene will not necessarily bring a cure, but by illuminating the fundamental processes of the disease it will open up new possibilities for treatment and diagnosis.

The intellectual challenge of the search is almost irresistible: to find a gene for which there is no clue as to its whereabouts or its basic biochemical defect—a task dismissed as impossible, or at least mad, even a decade ago. And to do so for a gene that would make such a difference.

"We're talking Nobel Prize material," says Robert K. Dresing, president of the Cystic Fibrosis Foundation, alluding to the rewards to the winner as well. Prizes aside, fame and glory—and probably generous research support—will belong to the winner, and, unfair as it may be, not to those who come in second.

And then there are the financial stakes, which are substantial. What sets cystic fibrosis apart from most other major genetic diseases, such as Duchenne muscular dystrophy (with an incidence of 1 in 5000 live male births) and Huntington's disease (1 in 20,000 births), are the vast profits to be made from diagnostic testings. For all three, finding the gene will enable improved prenatal diagnosis, a sizable market in itself. But for cystic fibrosis, finding the gene may also make possible widespread carrier testing—probably of the entire Caucasian population—a market that may be worth hundreds of millions of dollars a year. Not surprisingly, there is a hefty corporate interest in finding the gene.

Within a few months, it is generally believed, or at the outside a year, someone will find the cystic fibrosis gene. In London, Toronto, Boston, Ann Arbor, and elsewhere, labs are working flat-out, using the latest genetic techniques to home in on the gene, which has been localized to an increasingly small piece of chromosome 7. Williamson is very close to the gene, as is Lap-Chee Tsui in Toronto. But no one is talking about "candidate" genes anymore. They are keeping quiet and playing it close to the chest.

What makes all this possible in the first place is a new strategy for tracking down unknown genes, through a combination of molecular biology and classic cytogenetics, that was first suggested by Walter Bodmer and Ellen Solomon in 1979 and was then fleshed out in 1980 by David Botstein, Ray White, Mark Skolnick, and Ronald Davis.

Before that time, genes had been mapped to chromosomes by looking for the protein they make. But if the protein product was unknown, as it was for cystic fibrosis, Duchenne muscular dystrophy, Huntington's disease, and others, there was no way to distinguish that gene from the 100,000 or so others arrayed on the 23 chromosomes, and no way even to recognize the gene if by chance you stumbled upon it.

This new approach, which seems so simple in retrospect, is to use DNA markers to

get a fix on a gene's location. The key to this approach is the DNA markers, known as restriction fragment-length polymorphisms, or RFLPs. First detected in the 1970s, RFLPs are simply places on the chromosomes where the DNA sequence varies among individuals. RFLPs can thus serve as landmarks along the chromosomes.

Finding genes this way involves studying the DNA of families that carry a defective gene—say, the cystic fibrosis gene—to see if the disease trait is inherited along with any particular RFLP. If the disease is thus "linked" to the marker, the gene must be located on the same chromosome. And the closer the gene and the marker are on the chromosome, the less frequently they will be separated during the normal process of genetic recombination.

Thus, even with no hint as to the gene's identity, its location on the chromosome could be pinned down to within several million nucleotide bases or so. And once its chromosomal location is known, new molecular genetic techniques, such as chromosome "walking" or "jumping," can be used to pull out and clone the gene.

"Everyone read Botstein's classic paper," says Lap-Chee Tsui (pronounced "Choy"). He was a postdoc then in Manuel Buchwald's lab at the Hospital for Sick Children in Toronto, and he wanted to tackle cystic fibrosis but had no clue as to where to begin. "I read that paper and thought, 'Geez, this is so simple'."

Simple perhaps in theory, but not in practice. First they needed DNA markers (or more specifically, probes to detect the markers), which were scarce in those days. And they needed families for the pedigree analysis—large families with more than one sick child so that the contribution of each parental chromosome could be traced.

In Toronto, Tsui and Buchwald began looking first for families and then for markers. For families, they had an advantage: the hospital has one of the largest cystic fibrosis clinics in the world, so they were soon able to collect 50 families, with two or more living children with cystic fibrosis.

In London, Williamson and Kay Davies were already trying this approach on Duchenne muscular dystrophy, which looked like an easier target, at least to start, since the gene was known to reside on the X chromosome. At the same time, they were traveling around England seeking out cystic fibrosis families.

Other groups were doing the same thing, including Katherine Klinger at Case Western Reserve, Arthur Beaudet in Houston, and Anne Bowcock and Mary Claire King, who were working with L. Cavalli-Sforza in California.

At the same time, two other groups were amassing probes—the basic tools of linkage analysis: Ray White's group at the Howard Hughes Medical Institute at the University of Utah in Salt Lake City and Helen Donis-Keller's group at Collaborative Research Inc., a biotech firm in Bedford, Massachusetts, where Botstein was scientific adviser. The two groups had a different emphasis than the other researchers: to pursue Botstein's idea of creating a genetic linkage map of the entire genome, which would speed the search for any disease gene. Both would soon begin using this approach to seek out the cystic fibrosis gene as well.

By 1983 White was providing a sort of mail order service for the genetics community, sending probes to anyone who requested them. (Probes are no more than short, single-stranded pieces of DNA that can be used, much like a piece of velcro, to bind to and pull out a complementary piece of DNA on the chromosome.) Collaborative's collection of probes was actually larger than White's at the time, but the company was guarding it zealously for proprietary reasons.

Thus equipped with probes and markers, a handful of investigators set out to find the gene. But the first step, finding linkage between a probe and the gene, which would indicate which chromosome the gene was on, proved trickier than anyone imagined. They ran tens of probes, yet the gene remained elusive, unlike Duchenne muscular dystrophy or Huntington's, which yielded fairly quickly to these powerful new tools. Among all the groups frustration was beginning to mount. Indeed, some began to fear that cystic fibrosis might not be caused by a single gene defect after all, but rather might be caused by mutations of different genes.

To Tsui and Buchwald, it seemed like an unexpected windfall when, at the end of 1984, Collaborative approached them with a proposition: to pool Collaborative's many probes with the Toronto group's excellent family data to look for the gene. Tsui was working with 40 or 50 probes, and Collaborative had by that time amassed nearly 200. "Everyone wanted those probes," Tsui recalls. He would later realize that access to those probes did not come without a cost.

Collaborative's goal in pursuing genetic mapping in general and cystic fibrosis in particular was to develop diagnostic tests, which, they reasoned, would be enormously profitable—if this approach worked. The

A Hypothesis That Works

Cystic fibrosis is a recessive disease, and 1 in 20 Caucasians is a carrier. Most have no idea they harbor the defective gene until they unwittingly marry another carrier and pass on two copies of the defective gene to their child. After watching one child suffer through the disease, few carriers have additional children, choosing instead a type of genetic suicide.

Despite nearly a half-century of study, cystic fibrosis remains an enigma, its basic biochemical defect unknown. The puzzle is how a mutation in a single gene could account for the various manifestations of the disease—the thick mucus in the lungs, pancreatic dysfunction, and increased salt concentration in the sweat.

Now, for the first time, a hypothesis appears to be holding up to investigation. For years the salty sweat, which is used to diagnose the disease, suggested that the chloride channel, which transports chloride ions in and out of epithelial cells, might be involved. New work by Raymond Frizzell, Paul Quinton, and Rick Boucher now suggests that the chloride channel itself is functional in cystic fibrosis patients, but something is amiss in the gate that opens and closes the channel. Because of this defect, chloride is trapped within the cells. Excess sodium is also absorbed. This imbalance could explain the effects in the various organ systems.

Thus, the responsible gene probably codes for a protein in the membrane of epithelial cells involved in the gating process. When defective, the chloride channel is unable to open.

Gene replacement may be a possibility, but not for years, if ever. In the nearer term, once the defect is understood, it may be possible to correct its effects, or at least minimize them, pharmacologically. And short of that, it may be possible to alleviate the problem by augmenting the other pathways involved in chloride channel regulation, according to Robert Dresing of the Cystic Fibrosis Foundation. Opening the channel even partially might make a difference, says Dresing. "Cystic fibrosis need not be a lethal disease. Even some small augmentation may provide the kind of life we all want for these kids." ■ **L.R.**

same markers that help zero in on a gene can also be used in prenatal diagnosis in "informative" families to see if a fetus carries the disease, even before the gene has been found. Unable to convince investors of the wisdom of this risky new approach, the company had pumped nearly $10 million of its own money into genetic mapping. Now they were looking for a product.

By July Collaborative and the Toronto group had struck a deal: the company would send probes, the Toronto group would send cell lines, and both would simultaneously look for linkage. Whatever they found would be a codiscovery, though Collaborative would have first rights to any commercial product. Meanwhile, Collaborative had also approached White, but negotiations had bogged down.

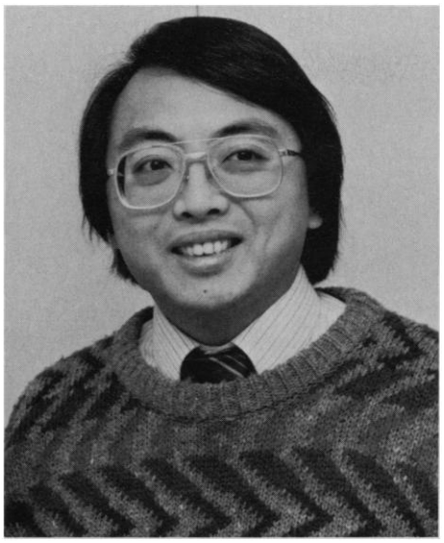
The first batch of a dozen probes arrived in Toronto in early August 1985 as Tsui was departing for Helsinki for a gene mapping meeting. It was there that the first break in the cystic fibrosis race was announced. Hans Eiberg of the University Institute of Medical Genetics in Copenhagen had found linkage with a protein marker known as PON. Because the protein is difficult to work with and its chromosomal location was unknown, it did not help narrow the search for the gene. Nonetheless, it did show they were on the right track, and that most, but not necessarily all, cases of cystic fibrosis must be caused by a single gene defect.

Meanwhile, unbeknownst to Tsui at the time, his lab had detected linkage as well, in the first test with a half-dozen of Collaborative's probes. The evidence was not conclusive—the probes had been run in only a small number of families, and the lod score, the geneticists' way of calculating odds, was 2.8, which gave them greater than 500 to 1 odds that they were on to something. A lod score of 3 (1000 to 1 odds) is considered proof of linkage.

As soon as Tsui returned from Helsinki his group tested the probe again in more families and came out with a score close to 4. It looked as if the probe was within 15 million bases of the gene—still a long stretch but closer than anyone had ever been.

On 26 August Tsui called Donis-Keller at Collaborative with the news. She was, understandably, skeptical; the company had expected to spend far longer screening probes. Two days later Collaborative scientist David Barker flew up to Toronto to go over the data. Skepticism vanished. It was fantastically lucky, but it was real.

But the most vital piece of information was still missing—what chromosome the probe was on—because Collaborative had not yet mapped it. Collaborative assured Tsui that they would do so right away; in



Lap-Chee Tsui: *"I think it is very clear, the company wanted to keep it a secret."*

fact, they had just set up a collaboration with Jean Frézal in Paris to map some of their probes. Tsui, Collaborative said pointedly, should not do the experiment.

"It was agreed that Collaborative and the French team would do the mapping and we would just sit here and wait and relax," says Tsui. "But that was a problem. We kept relaxing and relaxing."

On 18 September Tsui and Buchwald went to Boston. Collaborative still did not have the results. At that time Donis-Keller told them that preliminary work suggested that the probe mapped to chromosome 7, but the company was not sure—another chromosome was possible—and it would take 2 to 3 weeks to figure out. "Ordinarily, it would be a 1-week experiment," says Tsui.

Tsui was getting increasingly frustrated—and he was beginning to suspect that perhaps Collaborative was not telling him everything. This was the biggest break in his career, and he was supposed to sit by, knowing others were closing in on the gene.

"We weren't supposed to do the experiment, but we wanted to know so badly we did it anyway," he admits. "We couldn't sit here for a month and do nothing. You can imagine the anxiety."

The probe was on chromosome 7. "I was so happy it was on 7 I phoned Helen and said, 'Hey, we confirmed your result, it is on 7'." Donis-Keller, however, was far from elated. "She just blew her stack," Tsui says.

Tsui's problems were just beginning. The two groups decided to announce their finding in a paper in *Science* and at the American Society of Human Genetics in October in Utah, but they could not agree on what to report. The Toronto group wanted to report linkage to chromosome 7; Collaborative wanted to report linkage to an unmapped probe. "We were overruled," says Tsui. "I think it is very clear, the company wanted to keep it a secret."

Donis-Keller maintains that they genuinely did not know the location when they wrote the paper, and even by the time of the October Utah meeting, where they announced their findings, the data were not firm. "We were nervous about making a mistake. We wanted to feel certain it was on 7. It was very early for us; it was our first gene, and we were new to the field."

Tsui, on the other hand, suspects that Collaborative's strategy was to keep the location quiet until they could collect more markers on chromosome 7, closer to the gene. At a distance of 15 million bases, this first probe was too far from the gene to be useful in a diagnostic test. Finding closer probes would be relatively simple, now that they knew the gene was on chromosome 7. But by the same light, it would also be simple for their competitors. Meanwhile, the company filed a patent application on the first probe and any other probe between it and the gene.

But secrets are notoriously hard to keep in the tight-knit genetics community. At two meetings in Paris and Heidelberg in September, rumor was already out that the probe was on chromosome 7. Two of Tsui's competitors, White and Williamson, were at those meetings.

When Tsui arrived at the October Utah meeting, he found everyone was talking about cystic fibrosis—and specifically chromosome 7—but not in front of him. "It was very uncomfortable."

Tsui gave his talk, describing how they did the linkage analysis but not saying anything about location. Not surprisingly, the first question was, What chromosome is it on? Donis-Keller quickly responded: "We are working on it." "But at that time we already knew it was on 7," Tsui groans.

"It was almost a joke when Helen said she didn't know what chromosome it was on," says White. "All the interested parties already knew it was on 7."

"Anyone who says we withheld information is absolutely incorrect," says Donis-Keller. "We found linkage in the beginning of September. We wrote the patent application and the paper at the same time and submitted the paper on the 29th. I can remember the all-nighters we pulled. It wouldn't have been possible to write any faster."

Collaborative's behavior engendered considerable ill will among other researchers because of what they perceived to be the company's excessive secrecy and restrictive policies. Tensions were exacerbated by statements like chief executive officer Orrie

Friedman's "We own chromosome 7," which made scientists inside the company cringe as well.

"Lap-Chee is a hell of a nice guy," says Williamson. "He was caught in a terrible situation none of us had been in. I hope I never see that again—a company interfering in normal scientific communication at a very early stage, when there is no product involved and the work has not been done in house. There are some things you would expect a company to be proprietary about, but I did not see linkage that way. Linkage to cystic fibrosis is everyone's. You can't patent that. I think we all believe that."

White by that time had his own gripe with Collaborative. He thought the company was negotiating in good faith with him for a joint research effort on cystic fibrosis, although he admits that negotiations were going slowly, in large part because of problems at his end. The first White knew that negotiations were off was when he learned that Tsui and Collaborative had found linkage. Says White: "We felt misused."

But what the company's detractors rarely acknowledged was that Collaborative was following accepted commercial, if not academic, practices, and had invested $10 million in developing probes, which would later become a vast resource for the scientific community.

By all accounts, the company did not tread the line between scientific progress and profits very well, at least in the beginning. Since then, the company has learned, perhaps the hard way, how to forge better ties with the academic community. "Let's just say we learned," says Stanley Rose, the company's business development manager. "This is a developing field. Before we got into this business there weren't all these probes." Only now, as Collaborative institutes a more open policy to make those probes available, are tensions dissipating.

In early October 1985 Collaborative and the Toronto group were clearly ahead—they had won the first important victory in the hunt for the gene. But their lead would not last for long. Their competitors caught up—indeed, overtook them—within 2 months in a move that raised eyebrows even among the company's critics.

On 28 November 1985 three articles appeared in *Nature* reporting localization of the cystic fibrosis gene to chromosome 7: one by White, one by Williamson, and one by the Collaborative/Toronto team. While Collaborative's probe was so distant as to be essentially useless, White and Williamson's were very close.

White admits that he heard that Collaborative's probe was on chromosome 7 several weeks before the October meeting in Utah.

Sarah Putnam

Helen Donis-Keller: *"Anyone who says we withheld information is absolutely incorrect."*

The rumors were not entirely consistent, he says, but they were "good enough for us to reorder our priorities." And that they did, testing all the chromosome 7 probes they had access to. One of those was the ***met*** oncogene, which they had obtained from George Vande Woude at the National Cancer Institute. It turned out to be very tightly linked to the cystic fibrosis gene—within 1 million bases of the gene. His group submitted a paper to *Nature* on 1 November.

Williamson, on the other hand, denies that rumors played any part in his localization of the gene, which he submitted to *Nature* a few days later, on 4 November. By the time he heard rumors—and there were several conflicting rumors, he says—he was already confident that the gene was on chromosome 7 or 8.

What led the London group to chromosome 7, Williamson says, was the exclusion data, which indicate which chromosomes the cystic fibrosis gene could not be on. When various labs compared notes at the August Helsinki gene mapping meeting, he says, there were three obvious holes: chromosomes 7, 8, and 18. "Each group left Helsinki determined to test markers on those chromosomes," he says.

"That is not true," says Tsui. "The exclusion map was largely from the Toronto data," and they were not at all clear.

In a "personal account" of the search, published in the journal *Disease Markers*, Williamson gives only grudging precedence to the Toronto/Boston team. "Almost simultaneously three groups . . . found linkage to chromosomally assigned markers on chromosome 7q. The first convincing linkage was probably found by the Toronto group." As for the simultaneous publication, he calls it, "a fitting culmination of many years' work by all of those who were involved."

Others view it somewhat differently, including one of *Nature*'s reviewers, who characterizes White and Williamson's behavior as "immoral, but not criminal." Neither paper acknowledged the rumor or explained, at least convincingly, why they had focused on chromosome 7. The reviewer argued strongly that the papers should not be published without acknowledging the precedence of the Toronto/Boston group. White says that he was happy to acknowledge the rumor, which, in his view, represents no more than the "conventional passing along of scientific gossip."

The editors did not have long to ponder the dilemma. Tsui had already received a phone call from a colleague in Germany warning him about the White and Williamson papers. He was incensed. "Collaborative was still trying to hold onto the work. They were not publishing it." And now White and Williamson would get credit for what Tsui had done. "I was upset. I called Helen and asked her what she was going to do."

Collaborative called *Nature*, arguing that White and Williamson's work was based on rumor, and that Collaborative, though they had not yet published, had discovered linkage to chromosome 7. Collaborative threatened a major fight unless *Nature* published a paper by their team. Donis-Keller wrote the paper in 2 days. It was submitted on 11 November and accepted the next day.

When the papers appeared, they were accompanied with an unusual editorial comment, written by deputy editor Peter Newmark, providing the background that the papers omitted. Although Tsui and Collaborative did not announce localization at the October meeting in Utah, he wrote, "Nevertheless, rumor emerged that their RFLP was on chromosome 7 . . . although other rumors pointed toward other chromosomes. Spurred on by the rumors, two other groups have now found RFLPs that are linked to cystic fibrosis." The Collaborative/Toronto *Science* paper, reporting linkage to an unmapped probe, came out the following day, on 29 November 1985.

If the principals were sullied by such goings on, this could not dampen the excitement for what they accomplished: they were now within about 1 million base pairs of the gene—too far to "walk," or do so easily, but close enough to apply powerful new techniques to pull out the gene. And the scramble for scientific precedence was followed by a rare collaborative effort to speed the search for the gene. ■ LESLIE ROBERTS

Continued next week.