

The Promise of a Cure: 20 Years and Counting

The discovery of the cystic fibrosis gene brought big hopes for gene-based medicine; although a lot has been achieved over 2 decades, the payoff remains just around the corner

The gene hunt began quietly, with few theatrics and much uncertainty.

For Mitch Drumm, the starting gate lifted in the fall of 1985. He and geneticist Francis Collins met on opposite sides of a volleyball net, during a faculty-student mixer at the University of Michigan, Ann Arbor. Drumm, shorter than the lanky Collins, was outmatched in volleyball. But Collins quickly recruited Drumm to join the lab he was setting up, as its first graduate student. There, Drumm began experimenting with a gene-hunting technique Collins had developed. As a test case, they chose cystic fibrosis (CF), an inherited disease in

which sticky mucus accumulates in the lungs and elsewhere, eventually killing the patient. At the time, life expectancy hovered in the early 20s.

Coincidentally, CF had been on Drumm's mind. Just months before, the infant son of his family's next-door neighbors, close friends in New Philadelphia, Ohio, had been diagnosed with the disease. Drumm still recalls the phone call from his mother relaying the devastating news. Like many others studying CF, he became immersed in the field by a personal connection, which carried him through ups and downs in the decades ahead. A big triumph came nearly 4 years

All grown up. Danny Bessette, a 24-year-old with CF, was 4 years old when he appeared on the cover of *Science* announcing the discovery of the CF gene.

after signing up with Collins, in the spring of 1989. In collaboration with a large research group in Toronto, Canada, that had started an aggressive chase for the CF gene years earlier, the team cloned the CF gene—called the cystic fibrosis transmembrane conductance regulator (*CFTR*)—and nailed a crucial, disease-causing mutation (*Science*, 8 September 1989, pp. 1059, 1066, 1073).

Everyone in the CF community recalls the electric moment when they heard the news. “I remember seeing it roll off the fax machine, gathering people in the lab, and thinking, ‘What did we need to know’ ” now? says Michael Welsh, a pulmonary physician at the University of Iowa, Iowa City. Most believed that the disease had grown vastly less complex overnight and would soon be eliminated, probably by gene therapy.

On the 20th anniversary of the identification of the CF gene, as new gene discoveries pile up weekly and hype over the power of genes to transform medicine flows fast, CF offers an object lesson in how difficult it is, and how long it takes, to convert genetic knowledge into treatments. Every CF expert agrees that the gene discovery transformed their understanding of the disease's pathology. But even after so much hard work, not a single therapy based on the CF gene has reached the market. Some promising treatments, especially gene therapy, have proven bitterly disappointing.

“We were naïve,” says Johanna Rommens, who at the time was a postdoc in Lap-Chee Tsui's lab at the Hospital for Sick Children in Toronto, the counterpart to Collins's group in Michigan. In her 20s and relatively new to science back then, Rommens couldn't imagine a problem that defied resolution. “I thought I could do anything,” she says. “I sometimes feel discouraged that this was so hard.” Keen to experiment with other

CYSTIC FIBROSIS KEY DATES



1938: Physician Dorothy Hansine Andersen provides the first clinical description of cystic fibrosis.

1983: Chloride transport is identified as the major defect in CF.

1989: *CFTR*, the cystic fibrosis gene, is found. Median life expectancy for those with CF is about 29.



1990: Scientists suggest that protein folding is behind CF.

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genetic diseases, Rommens subsequently left the CF field.

Although gene therapy hasn't paid off, prospects have improved for those with CF: Their median life expectancy has stretched almost 10 more years and now exceeds 37. This is thanks not to genetic knowledge, however, but to more aggressive and earlier treatment to keep the lungs clear.

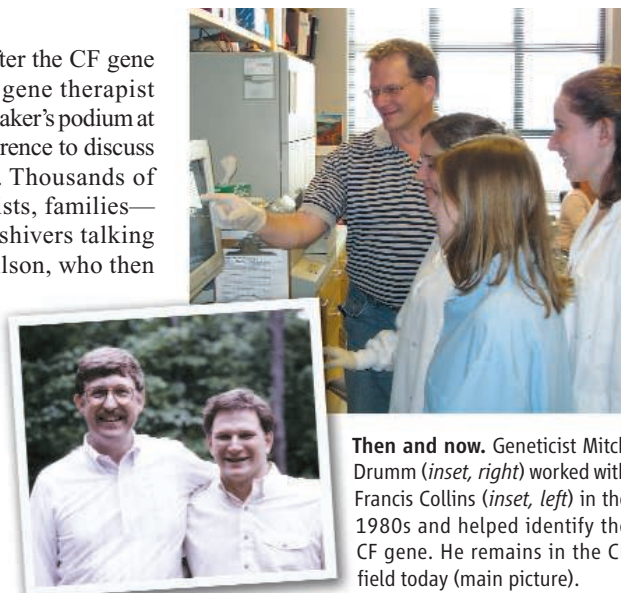
Soaring hopes

In October 1989, a month after the CF gene was published in *Science*, gene therapist James Wilson strode to the speaker's podium at a Florida cystic fibrosis conference to discuss prospects for gene therapy. Thousands of people—physicians, scientists, families—packed the meeting. “I get shivers talking about it right now,” says Wilson, who then worked down the hall from Collins at Michigan and is now at the University of Pennsylvania. “The excitement was palpable. I have never felt energy like that ever before.”

There was broad consensus that the time for CF gene therapy had arrived. Two advances buoyed hopes that this new technique, still in its infancy, would eliminate CF. First, scientists had managed to “cure” the disease in test tubes. They introduced a normal version of *CFTR* into cells from CF patients, compensating for a defective gene that produced no protein, or none the cell could use. In addition another researcher, W. French Anderson, then at the National Institutes of Health, began the first-ever clinical trial of gene therapy to treat an immune deficiency syndrome, demonstrating that gene transfer in people was feasible. By then, in the fall of 1990, says Wilson, “expectations for the success of gene therapy for CF were as high as I've ever seen for any disease, under any circumstances, in the 20 years I've been involved in this.”

Looking back, many CF experts consider

an excessive focus on gene therapy in the early years to have been a big mistake. Like an investor who gambles much of his or her fortune on a single stock, “people kind of stopped doing the other things they were doing” and turned instead to strategies for getting the gene into lung cells, says Raymond Frizzell, a physiologist at the University of Pittsburgh in Pennsylvania.



Then and now. Geneticist Mitch Drumm (*inset, right*) worked with Francis Collins (*inset, left*) in the 1980s and helped identify the CF gene. He remains in the CF field today (main picture).

Early successes in basic CF research also convinced scientists that new treatments were right around the corner. In addition to correcting the gene defect in cells, having *CFTR* in hand revealed that the healthy *CFTR* protein was an ion channel that helped transport chloride and control the movement of water across cell membranes. Researchers determined that several mutations led to a protein-folding defect—one of the first protein-folding diseases identified. Then in 1992, Welsh and his colleagues published a paper in *Nature* explaining that the protein-folding problem could be corrected by chilling the cells. Although this tactic wasn't useable in patients, it allowed “people to say, ‘It's misfolded but you can overcome that,’” Welsh says.

Even with these heartening advances in the early 1990s, there were hints that choppy waters lay ahead. The CF protein was a bear to work with because it didn't respond well to classic analytical tools like Western blots and antibody assays, recalls Margarida Amaral, now on sabbatical at the European Molecular Biology Laboratory in Heidelberg, Germany, who worked on the protein at the University of Lisbon in Portugal. “Nothing seemed to work.”

In Wilson's lab, meanwhile, postdoc John Engelhardt, now at the University of Iowa, was running into difficulties getting gene therapy to work. The gene's expression varied wildly depending on which parts of the lung researchers examined. One great appeal of gene therapy for CF was that a vector carrying a working CF gene could easily be introduced into the lung with aerosols. But Engelhardt found that only about 1% of cells lining the lung's airway—the cells that come into contact with aerosols—boasted high levels of CFTR protein.

Each advance provoked more questions. When Richard Boucher, an adult pulmonologist at the University of North Carolina, Chapel Hill, won a three-way race to create the first mouse model of CF in 1992, he and others were dismayed to find that the rodents didn't mimic human disease. They shared the gut afflictions of CF patients, who must take pancreatic enzymes for life to break down thick secretions. But the lungs of CF mice, unlike those of their human counterparts, were healthy.

Like Drumm, Boucher traces his passion for CF to a personal experience: His daughter suffered several bouts of pneumonia as a baby and was suspected of having CF. Panicked, he read up on the disease; this drew him to a career teasing apart its mysteries. The CF mouse was a big one: Why were its lungs clear? Boucher and others determined that the animals had a second chloride channel that was unaffected by CF. This led to a new understanding of how the airway surfaces stayed healthy: As long as chloride could pass

1990:

Patients with the same gene mutation don't share the same severe disease, suggesting a role for other genes or the environment.



1992:

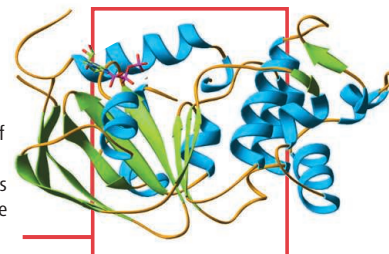
Scientists describe the first mouse model of CF, but the mice don't have lung disease.

1993:

A 23-year-old man with CF receives the first dose of gene therapy.

1993:

The number of mutations in *CFTR* surpasses 200. Today, we know of over 1500 different mutations.



through, the lungs fared well. But in other ways, the mouse proved of virtually no value. It would be 15 years before other researchers found a better animal model.

Meanwhile, gene therapy plowed ahead. In 1993, a 23-year-old became the first CF patient to receive a dose of healthy *CFTR* by gene transfer. Three small clinical trials began: one led by Wilson, one by Welsh, and one by Ronald Crystal at Weill Cornell Medical College in New York City. “Boy, there were all kinds of issues,” Wilson recalls. Among them: potent fears that the virus carrying healthy *CFTR* into a patient’s nasal passages or lungs would recombine with another virus, be shed by that patient, and “create an environmental catastrophe.” Volunteers in the trials were put in strict isolation.

The bigger issue, as it turned out, was that gene therapy simply didn’t work. Few lung cells took up the gene. There were also concerns about inflammation, as the lung rebelled against a viral intruder. “You had to confront the reality of eons of evolution” that had built barriers against toxins and infections, says Boucher, who also worked in CF gene therapy. Researchers in the United States spent several more years trying to get around this, tinkering with gene therapy in baboons, rhesus macaques, and other animals, before largely giving up.

Shifting gears

Although the trials failed to help CF patients, they mattered to clinical research: For the first time, viral vectors were injected directly into a patient (as opposed to affected cells being removed, modified, and reinfused), and this became the new model for a nascent specialty. The CF trials also underscored the problem of immune reactions to treatment, which hadn’t previously been appreciated, says Wilson.

In a funny way, “science has benefited more from the CF gene than CF has benefited from the science,” says John Riordan, a bio-

chemist and, with Tsui and Collins, one of the co-discoverers of the CF gene when he worked at the Hospital for Sick Children. Now at the University of North Carolina, Chapel Hill, Riordan never thought he’d still be working on CF 20 years later. But he points out that *CFTR*, which belongs to a large family of membrane proteins, is unusual, using several different mechanisms to carry out its func-

tions. As the years passed, biologists studying *CFTR* learned much about how chloride is transported across cells, and that the protein may also influence inflammation, cell signaling, and other processes. They

found that cells build complexes of *CFTR* and other proteins to keep the system humming.

But what about a cure for this genetic disease, for which there’d been such high hopes? By 1998 or so, researchers knew far more about CF than they had 10 years before. They knew, for example, that hundreds of different mutations in *CFTR* could cause the disease and that not all disabled the protein in the same way—suggesting that different treatments might be needed for different patients. They knew, too, that *CFTR* couldn’t explain everything. Some severely affected 12-year-olds needed lung transplants, and some 28-year-olds were running marathons—even when the quirk in their *CFTR* gene was identical. This led researchers to consider that other genes also play a role in CF, as do environmental factors.

None of this was quickly leading to new treatments. “1997, 1998 was really the point where we said, ‘Academics are great, but if we really want to discover drugs, we’ve got to become more businesslike,’” says Robert Beall, president and CEO of the Cystic Fibrosis Foundation. The CF Foundation had been instrumental in funding the gene hunt and subsequent CF research, raising and investing tens of millions of dollars. In the late 1990s, Beall began shopping around plans to develop

small-molecule, traditional drugs—back to basics after gene therapy had failed.

“A lot of people thought that Bob Beall was going far out on a limb to put a lot of money into a strategy that was clearly risky,” says Collins. Multiple drugs might be needed to tackle different *CFTR* defects. Companies apparently were wary, too. Beall telephoned seven; two called back. One was Aurora Biosciences in San Diego, California, which was bought by Vertex Pharmaceuticals in 2001. It agreed, with generous support from the CF Foundation, to see what it could do.

Progress at last

More than \$75 million and another 10 years later, two Vertex drugs are taking the CF world by storm. One, VX-809, is designed for the most common CF mutation and helps *CFTR* get to the surface of the cell. Only safety data are available on VX-809 so far.

The other Vertex drug, VX-770, aims to boost the function of *CFTR* protein that’s already made its way to the cell surface—which would help in at least one of the CF mutations, accounting for a few percent of cases of the disease. Last October, Vertex reported that in a phase II trial, lung function of volunteers improved by 12% in 4 weeks of treatment. “That’s more than any drug ever improved the disease” in any span of time, says Beall. The excitement around Vertex is so great that at a recent CF fundraising walk, organizers gave two Vertex employees the bib numbers 770 and 809, says Paul Negulescu, a vice president of research at the company’s San

Diego office. And everyone knew what those numbers meant.

The CF field has enjoyed other recent breakthroughs. In September 2008, Welsh and his colleagues described a CF pig model in *Science*—the first animal model that closely resembles human CF. More recently at Iowa, Engelhardt, who worked in Wilson’s lab in the old days and also collaborated on the pig, has developed a CF ferret, the culmi-

“We have miles to go before we sleep.”

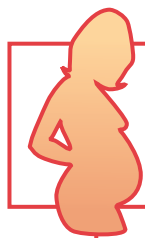
—PAUL QUINTON,
UC SAN DIEGO

Online sciencemag.org

Podcast interview
with author
Jennifer Couzin-Frankel.



1995:
Gene therapy fails to help CF patients.



2001:
Two medical professional groups recommend prenatal CF carrier screening.

2005:
Researchers show that the gene TGFβ modifies the severity of CF.

2006:
The first drug designed to target a CF protein defect, by Vertex Pharmaceuticals, enters clinical trials.



2008:
Pigs become the second animal model of CF.

nation of 10 years' work. (The group spent more than 2 years just trying to understand ferret lung biology.) "Not having good animal models has really slowed the field down," says Engelhardt. Therapies can have harmful side effects, so "when you think about treating a kid before they have overt disease, you've got to be pretty sure you've got a great treatment." Testing in animals offers some reassurance.

Gene therapy, too, is experiencing a resurgence. In the United Kingdom, a team of 80 investigators is launching a 100-person trial using fat particles—unlike the viral vectors in earlier U.S. studies—to carry *CFTR* to cells. The U.K.'s Cystic Fibrosis Trust has dedicated considerable effort, and \$50 million, to gene therapy. "Someone needs to find out" if this works, says Eric Alton, a gene therapist at Imperial College London who's heading up the trial, which he hopes will reveal how distant the goal is. "We're either sitting on the therapy, or we're a million miles away from it." Despite earlier setbacks, Alton still feels that gene therapy offers more hope than drugs, because in theory at least, it's more comprehensive. Researchers have found at least 20 functions for the CFTR protein. A drug can correct only one or two at once—whereas gene transfer, if it works, can do it all. Results from Alton's trial, which is just beginning, will come in 2012.

There have been other developments: Prenatal testing is increasingly offered to couples contemplating pregnancy, potentially reducing the number of babies born with CF, although figures are difficult to come by. Forty-seven states and many countries now test newborns for CF, enabling treatment to start right away rather than months or years later, when a child fails to thrive.

Humbling science

Many CF experts say that, after 20 long, frustrating years, it's possible now, finally, to look patients in the eye and assure them that in a few years, treatment will be vastly improved. And patients are optimistic, too. "I can't imagine where we're going to be in another

25 years if it's not cured," says Ryan Ress, a 24-year-old with CF. Ress was Drumm's infant next-door neighbor who inspired the geneticist, now at Case Western Reserve University in Cleveland, Ohio, to stick with CF, and the two remain in touch. Ress majored in biochemistry in college and spent a summer working in a CF lab next door to Drumm's. He's now studying to be a neonatal nurse practitioner. Ress is convinced that CF will be



Needle in a haystack. Capsules, each containing DNA that might harbor the CF gene, filled the freezer in Lap-Chee Tsui's lab in Toronto.

conquered, based on his reading of the disease literature and the belief that "there will be a reward" for CF researchers for their backbreaking years of work.

But with lessons learned the hard way, caution abounds, too: "We have miles to go before we sleep," says Paul Quinton, a physiologist at the University of California, San Diego. Quinton is a rare bird. At 20, in college and thinking about his own mortality, he says, he began combing through textbooks in his campus library, hunting for an explanation for the abdominal troubles, chronic cough, and lung problems that had plagued him for years. In books he found an answer: CF. Soon after, Quinton abandoned his dream to become a poet and turned to understanding his disease. In 1983, he deter-

mined that chloride transport was the fundamental defect in CF, one of the biggest breakthroughs in the field.

Quinton learned via genetic testing that he harbors one severe mutation and one that's milder, a combination that may explain why he's survived as long as he has. Now 64, he admits that he was as optimistic as the next person when the CF gene was found, even declaring in an editorial in *Nature* that the chance to cure CF had become reality. These days, though, he sees questions everywhere. How, exactly, does normal CFTR function? How does the absence of CFTR lead to the thick mucus of CF? Will even today's most promising drugs work in more than a very narrow slice of patients?

The case of CF, agrees Amaral, is "a lesson in being humble in science."

What does this mean for the flood of genes identified in the years since—both for single-gene diseases and more complex ailments? One shouldn't generalize from the CF story, says the irrepressibly optimistic Collins, former director of the National Human Genome Research Institute, because every disease is different. He knows of at least one—progeria, which causes accelerated aging—in which a gene he helped identify led to a drug within 5 years that's now being tested in nearly every child with the disease.

Collins's early competitor and later collaborator in the CF gene hunt, Tsui, treads more carefully. "Because of the excitement, some scientists, perhaps even disease funding agencies, ... wanted to give people hope, or give themselves hope," says Tsui, who in 2002 left Toronto to become vice-chancellor and president of the University of Hong Kong. "They were a little bit optimistic at predicting when a cure would be there. ... [It] taught a lesson to other gene researchers." Namely: don't spin prophecies, don't assume that the gene is the end of the story. Rather, it's just the end of the beginning, with a long road still ahead.

—JENNIFER COUZIN-FRANKEL

2008:

Forty-seven U.S. states are screening newborns for CF.



2008:

The United Kingdom launches a new CF gene therapy trial.

2009:

A gene for type 2 diabetes predisposes children to CF-associated diabetes.

2009:

The phase 3 clinical trial for one Vertex drug opens after lung function improved in an earlier study.

2009:

Median life expectancy for those with CF exceeds 37 years old, thanks to more aggressive and earlier treatment.

