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ONE GENE, TWENTY YEARS

When the cystic fibrosis gene was found in 1989, therapy seemed around the corner. Two decades on, biologists still have a long way to go, finds **Helen Pearson**.

During the day, Lap-Chee Tsui and Francis Collins were attending a gene-mapping workshop. At night they were scrutinizing the pages churning out of a fax machine they had set up in a dorm room. Their hunt for the cause of cystic fibrosis had reached a gene that looked from its sequence like it might have a role in transporting ions through cell membranes, a process that goes awry in those with the disease. The fax they received that night from Tsui's lab showed that many people who have cystic fibrosis lack three base pairs from both copies of this gene, whereas those without the disease always have at least one copy intact. With that fax, on a rainy night in May 1989, "I was convinced — that was the moment," Collins says.

Four months later a four-year-old boy with cystic fibrosis, Danny Bessette, was shown sitting cross-legged on the cover of *Science*, framed by a rainbow of chromosomes. Inside the magazine, three papers[1–3] laid out the details of the discovery of the gene responsible for Bessette's condition — the first gene for a human disease discovered without the help of an already-known protein sequence or any clue to its whereabouts. "In this issue ... there is a story that does not begin at the beginning or end at the end, but has a very happy middle," wrote *Science*'s editor Daniel Koshland[4]. "One in 2000 children born each year with a fatal defect now has a greater chance for a happy future." By that stage, news of the finding had already leaked to the media, been the subject of two hastily assembled press conferences and been trumpeted in newspapers worldwide. "It would be difficult to overstate the importance of the cloning of the cystic fibrosis gene," wrote geneticist Peter Goodfellow in *Nature* that month[5]. "The implications of this research are profound: there will be large spin offs in basic biology, especially in cell physiology, but the largest impact will be medical."

So far, Goodfellow's prediction has proved wrong, at least as far as medical impact is concerned. As Jack Riordan, who collaborated with Tsui and Collins on the original discovery, puts it: "The disease has contributed much more to science than science has contributed to the disease."

This is not to deny that medical progress has been impressive. An American born with cystic fibrosis today has a life expectancy at least ten years longer than one born in 1989 did. Such advancements help explain why Bessette — now 24, and pictured opposite — has a future at all. But many researchers concede that relatively little of that improvement can be laid at the door of the cystic-fibrosis transmembrane regulator gene, or *CFTR*. Gene therapy — the source of so much of the hope in 1989 — has so far bought no one with this condition a single additional year of life; no therapies targeted at the CFTR protein have yet been approved. Researchers have not even fully agreed on a hypothesis to explain how mutations in the gene cause the condition. But the gene itself "found its way into all departments", says Riordan, leading to progress in fields as diverse as protein trafficking and membrane transport. And the gene-hunting techniques that Tsui, Collins, Riordan and their colleagues pioneered have laid the foundation for a genetic understanding of all human disease.

"The disease has contributed much more to science than science has contributed to the disease."
— Jack Riordan

Twenty years, although a long time in the life of a young man such as Bessette, is not the whole story. Several hundred million dollars have been spent trying to find a therapy that directly tackles the molecular defects that underlie cystic fibrosis; Collins, for one, thinks that this means the hopes on which gene therapy never delivered are about to be fulfilled. Like many researchers, he is excited by clinical results coming through on a pair of small molecules that could get mutant versions of the CFTR protein to work properly. Should the molecules be approved, "it will be a pair of home runs, a milestone for all genetic disease", Collins says. And those home runs would never have been hit without the gene and the opportunity to study the protein that needs fixing. "You can paint a direct pathway from the gene discovery [to those drugs]," he says.

To call the path direct might be overstating it. Researchers have taken many paths from *CFTR*, and their travels have shown that behind this gene and every one found since lie dauntingly complex biological stories. "I think one of the lessons of cystic fibrosis is the recognition of the enormous challenge that faces us in human biology," says Riordan, now at the University of North Carolina, Chapel Hill. "It's not like going to the Moon — it's going to Mars." The size of the challenge can sap enthusiasm. "Looking back, it was an important contribution," says Tsui, "but I'm disappointed because at this time, from my own research, I was not able to help very much." Riordan says that he now views "the latest hot gene" with a "jaundiced eye". But one thing that shines through when speaking to these three and other researchers is their continued optimism, their passion and their sense of urgency. "Perhaps," says Collins, "we've taken our blinkers off. Perhaps we couldn't deal with it before, and now we have a lot more tools to dissect the complexity."

"It's not that it hasn't worked," says Riordan. "It's only been 20 years."

Blind beginnings

Geneticists have been interested in cystic fibrosis since the disease was first identified in the 1930s. The disease is common in Caucasian populations — about 1 in every 25 people carries a mutated copy — and its pattern of inheritance is straightforwardly Mendelian: those with one mutated gene are healthy carriers; those who inherit two will have the condition. Doctors knew that although the pancreas often fails and the gut is unable to absorb nutrients, the lung is the organ that is crippled with recurrent and persistent infections, "and that's unfortunately the one that kills them", says Richard Boucher, a pulmonary physician and cystic fibrosis researcher at the University of North Carolina. But for

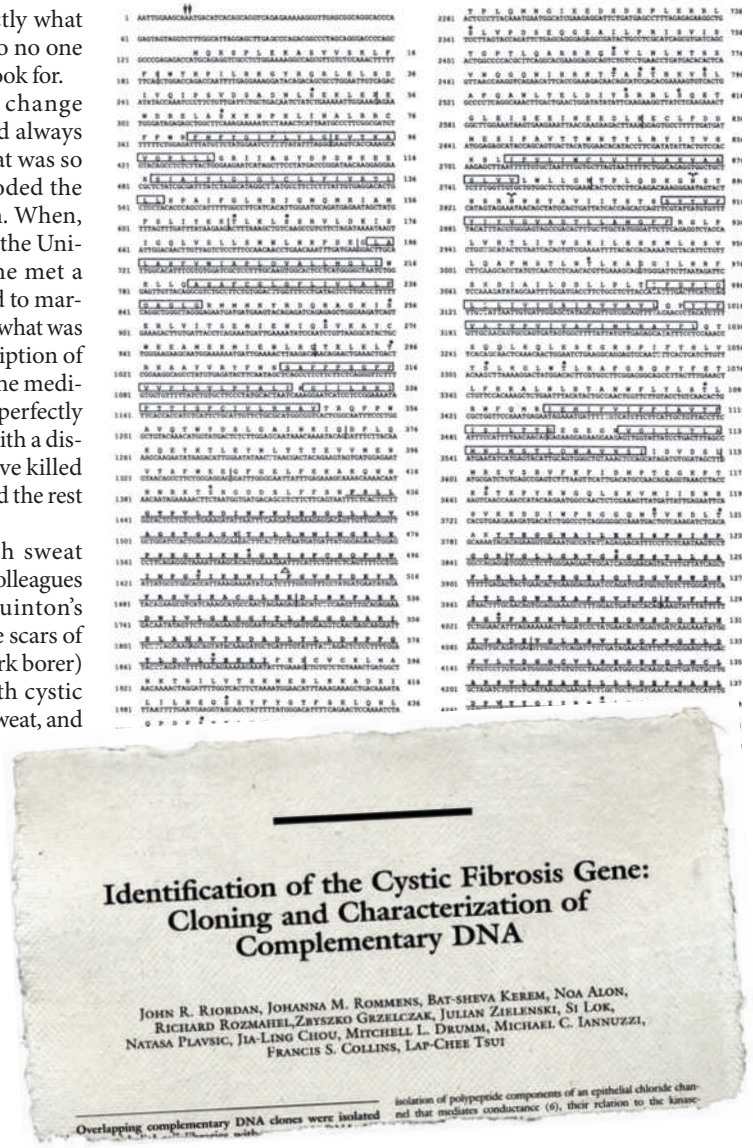
J. LUIS MAGANA/AP

decades no one knew exactly what was wrong with the cells, so no one knew what type of gene to look for.

Paul Quinton helped change that. As a kid, Quinton had always coughed a lot, and his sweat was so salty that his clothes corroded the wire hangers they dried on. When, in 1965, as a 19-year-old at the University of Austin, Texas, he met a girl and his thoughts turned to marriage, he decided to find out what was wrong with him. The description of cystic fibrosis he found in the medical library fit his symptoms perfectly and he diagnosed himself with a disease that should already have killed him, but that he would spend the rest of his life studying.

Quinton collected fresh sweat glands from visitors, from colleagues (Riordan, who visited Quinton's ranch, says he still bears the scars of Quinton's biopsies with a cork borer) and from other people with cystic fibrosis to explore why his sweat, and that of others with the disease, was so salty. In 1982, while working at the University of California, Riverside, an experiment measuring the ability of sodium and chloride to pass through the glands led him to finger a channel that was unable to conduct chloride ions across the epithelium of the skin, and that might also underlie problems in the lungs and the other affected organs[6]. "I feel silly saying it but I literally jumped up and ran up and down the hall shouting 'Eureka'," says Quinton, who now also works at the University of California, San Diego. "I still get chills; it was one of those moments you get once in a lifetime." The disease is evident when he speaks: he still clears his throat and coughs a lot.

Quinton's discovery and others like it told geneticists what they should be looking for: a gene that is involved in the movement of chloride, and perhaps other ions, across the epithelium. By now an intense and competitive hunt was under way. It was the 1980s, when the human genetic sequence was largely uncharted territory, and the human genome project was still a twinkle in various eyes, including Collins's. Finding the gene would be a technical and intellectual challenge as well as a medical breakthrough. Until that point, almost all of the genes that had been associated with human diseases had been identified by first isolating the protein responsible. A protein's amino-acid sequence reveals much of the gene's probable nucleotide sequence, and that made pinpointing the gene easier. The few exceptions, such as those found for Duchenne muscular dystrophy and retinoblastoma, were helped by a few patients with chromosomal abnormalities that pointed to the gene's position. For cystic fibrosis, researchers were working blind: they had no protein and no location. This was to be a big test of new 'reverse genetics' techniques, in which a gene is found by searching for markers in the genome that are consistently inherited with the disease in affected families and using them as signposts to the gene itself.



Identification of the Cystic Fibrosis Gene: Cloning and Characterization of Complementary DNA

John R. Riordan, Johanna M. Rommens, Bat-sheva Kerem, Noa Alon, Richard Rozmahel, Zbyszko Grzelczak, Julian Zielenski, Si Lok, Natasa Plavsic, Jia-Ling Chou, Mitchell L. Drumm, Michael C. Iannuzzi, Francis S. Collins, Lap-Chee Tsui

Overlapping complementary DNA clones were isolated ... isolation of polypeptide components of an epithelial chloride channel that mediates conductance (6), their relation to the kinase-

The sequence of *CFTR* revealed the position at which three base pairs are commonly deleted (triangle).

Tsui, then at the Hospital for Sick Children in Toronto, Canada, and now at the University of Hong Kong, was a key player in the hunt; so were Robert Williamson at St Mary's Hospital Medical School in London, and a handful of other researchers. By 1985, several groups[7–9] had shown that the gene mapped to a region of chromosome seven, but it was still a vast genetic wilderness somewhere between one and two million base pairs wide. In 1987, Williamson announced that he had landed on the gene, but soon after had to admit he had got it wrong. Nevertheless, many groups assumed that Williamson was close and dropped out of the race at that point. Says Collins: "Lap-Chee and I were more stubborn".

Collins, then at the University of Michigan in Ann Arbor and until last year the head of the National Human Genome Research Institute in Bethesda, Maryland, met Tsui at that year's meeeting of the American Society of Human Genetics. A few years previously, Collins had described the technique of 'chromosome jumping', a way of leaping across the vast genetic distances from one marker sequence in a region to another that was much faster than the conventional way of chromosome 'walking'[10,11]. They agreed to collaborate: Collins's lab would bound to new positions, and Tsui's would walk forwards and backwards from the landing points looking for the gene. Two years later, on that rainy night in the dorm room, their fax machine told them they had found it.

The *Science* papers showed that the gene looked like others encoding membrane proteins that transport ions. The three base pairs missing in the vast majority of people with cystic fibrosis eliminated an amino acid at position 508 of the protein's amino-acid sequence, a mutation called ΔF508. "It was exciting times," says Robert Beall, then executive vice-president for medical affairs at the Cystic Fibrosis Foundation in Bethesda, Maryland, and now its director. "We had been at a bottleneck. We didn't know why chloride wasn't getting out of cells, and that gene solved it."

The scramble of competition continued as researchers

rushed to work with the gene. John Hanrahan at McGill University in Montreal, Quebec, recalls the time he was collaborating with Riordan on a paper for *Cell*[12]. Riordan called him to ask him to fax through a figure for the manuscript as he was worried about a scoop from a competing paper at *Science*. "I raced to the airport in a snowstorm to send the originals by same-day courier, but it was the faxed version that went to press," Hanrahan says. "When people look at the traces they must wonder, 'Why are they so pixelated?'" But Hanrahan, like most researchers, says that the competition was a healthy one, even if it deprived them of a little sleep. "I think a lot of data were published and some mistakes were made, but there was tremendous excitement and the field moved ahead rapidly."

From the beginning, the goal was gene therapy. Get a good gene into the patients and they would make the proper protein; with the proper protein they'd be cured. But the path from gene to therapy wasn't smooth. It took more than a year just to get bacteria to produce the protein from the cloned gene, because of 'cryptic' sequences within the gene that prevented the bacteria from expressing it. But by 1993 the first clinical trials were under way.

"The expectation was that all you needed to do was get a little bit of stuff to act in the lungs and 'hey presto' you'd have a Nobel prize," says Steven Hyde, who works on cystic fibrosis gene therapy at the University of Oxford, UK. Among other things, the lung, researchers now realize, is just about the worst possible target for such an approach. Its sophisticated defences against infection have evolved precisely to prevent the sort of uptake and expression of foreign material the gene therapists were after. Mike Welsh at the University of Iowa and his colleagues, who in one of the first trials pushed the gene into cells in the nasal passage as a surrogate for those in the lung, later realized that the cells that had taken up the gene were probably damaged during the procedure. "A whole slew of people did similar trials and everyone got a little disillusioned." Hyde says.

Disillusionment isn't enough to kill off an idea — but death is. In 1999, a severe immunological reaction killed Jesse Gelsinger in a gene-therapy trial for an inherited liver disease, casting a pall over the entire field. In the United States, the field has never fully recovered. In other countries — the United Kingdom and France, for example — researchers have been much more active in pursuing the technique.

Around the same time, Beall decided to turn the gene into a way to find a therapy, rather than being the therapy itself. He wanted to take advantage of new tools coming online for high-throughput drug screening. Researchers inserted the gene into cells, expressed the mutated protein, then screened for drugs that could correct the way the protein is made or the way it works. "People thought we were crazy," Beall says. What started as US$2-million grant in 1999 has turned into an $76-million programme, and Beall proudly points to a chart showing the drugs working their way through the pipeline as a result.

By far the most common mutation is ΔF508. It causes the protein to fold up poorly, and a drug known as a corrector is needed to help it fold correctly and get to the membrane it needs to sit in. Other mutations — there are now more than 1,500 known in the gene — require different approaches. Versions of the gene in which protein translation stops short need drugs to override the stop signal. Then there are proteins that get made, fold up and reach the membrane but just don't work properly. They need what are called potentiators.

Lap-Chee Tsui, Francis Collins and Jack Riordan (left to right) celebrate their 1989 discovery of the cystic fibrosis gene with a patient.

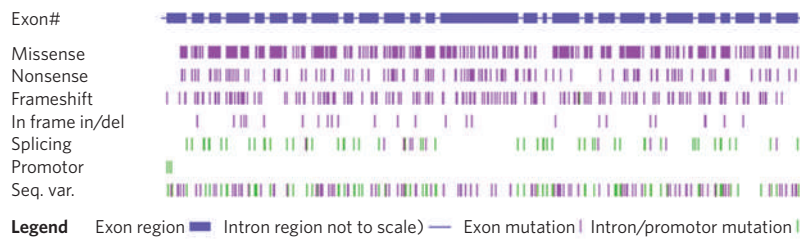
CANADIAN CYSTIC FIBROSIS FOUNDATION

"Perhaps we couldn't deal with it before, and now we have a lot more tools to dissect the complexity."
— Francis Collins

In March 2008, investigators presented results from a phase II trial of the potentiator VX-770 to a room of several hundred researchers at a meeting of the Cystic Fibrosis Foundation. Just two weeks of treatment in 20 people with a rare mutation called G551D had dramatically lowered some people's sweat chloride and produced some improvement in lung function — something that clinicians found particularly remarkable given the battered state of their airways. "When they showed those data and I saw the emotions from those physicians, it was unbelievable," Beall says. "It was the most emotional time since the discovery of that gene. It's telling you we can change the course of this disease." Collins agrees. "It was wildly better than even the most optimistic perspective for a small-molecule trial," he says. Phase III trials of VX-770, developed by Vertex Pharmaceuticals of Cambridge, Massachusetts, are now recruiting patients. Beall and others say that the drug might find a much wider market if it is also used in people with other mutations, including ΔF508, in conjunction with a corrector. That corrector could be another Vertex drug called VX-809, which is just starting phase II trials.

A special case

Must it take 20 years to get from gene to drug? No. Various things have made cystic fibrosis peculiarly difficult. One has been a lack of a complete understanding of how the CFTR protein leads to the disease. Many think that the defective channel causes the lungs to absorb too much water; others have argued that the primary problem is an incorrect ion



More than 1,500 mutations of various types have been found in the *CFTR* gene.

composition that disables the lungs' normal defences against infection. This debate became so fierce it was described as the 'salt wars'. At least part of the problem seems to lie in another ion channel that CFTR interacts with. "If you ask 20 people you'll get 20 different hypotheses," says Welsh. "Everybody's got their favourite — I think we don't know."

Then there are some purely technical problems. Mice with mutated versions of *CFTR* have few obvious lung problems and thus make poor models of the disease. (The models have, however, revealed something about why the mutated gene is so common — see 'A killer advantage'.) The CFTR protein is huge and is embedded in a membrane, making its structure difficult to determine with X-ray crystallography; plus the fact that airway cells tend to contain only a hundred or so copies of the protein, so there is very little of the stuff to play with. Together, these mean that no one has been able to resolve a complete high-resolution structure for the protein, which has hampered understanding of how it works and the design of drugs.

Other genes have had it easier. Collins points to the gene for Hutchinson–Gilford Progeria Syndrome (HGPS), an extremely rare single-gene disease that causes young children to shows signs of old age. The gene was discovered by Collins's team at the National Human Genome Research Institute in 2003 (ref. 13) and by another group in France[14], and a treatment based on it went into a phase II clinical trial in 2007 — a notably fast pace of translation. Collins puts much of the speed down to serendipity. The mutated protein was an extremely well-studied one called lamin A, and a cancer drug that had already reached late-stage clinical trials was found to work against the mutated protein, saving some laborious drug screening and safety testing. What's more, the task required of the drug is simpler. Drugs for cystic fibrosis have to compensate for or restore the function of a mutated protein, whereas those for HGPS simply have to block the action of one that has turned toxic.

"If you ask 20 people you'll get 20 different hypotheses. Everybody's got their favourite."
— Mike Welsh

The discovery of *CFTR* deserves at least some credit in the HGPS story, though, as it does for accelerating the pace of translation after almost every gene discovery since 1989. That's because hard work and mistakes made in this field have saved effort in every other. "If you found a new gene tomorrow you could compress those 20 years hugely because of what's been done with cystic fibrosis," says Hyde.

And gene therapy may yet prove possible for cystic fibrosis. In 2001, the Cystic Fibrosis Trust in Bromley, UK, asked Hyde's group and two others in Britain that were still working in the field to stop competing and start working together. They complied and have spent several years and around £30 million (US$49 million) working methodically through some of the problems — such as devising better ways to measure changes in lung function. Earlier this year, researchers at Imperial College London treated the first of 27 people with cystic fibrosis in what is expected to become the largest gene-therapy trial ever undertaken for the disease. The aim is to test whether the gene can be delivered safely, in a fatty particle called a liposome. If it is, the researchers will scale up to a 100-person randomized controlled trial to see whether it is effective. "I think we have now tempered the optimism of the early 90s with a heavy dose of realism," says Eric Alton, who directs the trial.

Clinical changes

Throughout this time there have been dramatic changes in the way that cystic fibrosis is treated in the clinic. In 1994, Genentech introduced Pulmozyme (dornase alfa), an enzyme that breaks up some of the lung-clogging mucus that encourages infections. A few years later, aerosolized antibiotics were introduced to fight these infections more aggressively. Earlier this decade doctors in Australia started noticing that their patients who surfed felt better during the surf season — leading researchers to test the idea that the daily inhalation of super-salty water, called hypertonic saline, could help lubricate the lungs. It did[15,16], and this is now standard therapy for many patients. Not all of them benefit from this approach, though: Bessette stopped taking hypertonic saline after a few years because it made him cough blood from a burst vessel in his lung. Pulmozyme does

A killer advantage

Work with the cystic-fibrosis transmembrane regulator gene, or *CFTR*, has helped to solve one puzzle about the disease: why do so many people carry a mutated version? For most of human history, people who inherited two mutated copies died long before they reached reproductive age — and if they didn't, men with cystic fibrosis tend to be infertile because they lack the sperm-carrying vas deferens. This means that selection should long ago have weeded out the mutations. The fact that it has not has led to the hypothesis that the mutated gene has a 'heterozygous advantage': people who carry one copy (heterozygotes) have some selective advantage that keeps the frequency of mutated alleles high.

One idea, that heterozygotes might enjoy some kind of increased fertility, has prompted numerous studies but most of them have been inconclusive. Then, in 1988, geneticist Lynn Jorde from the University of Utah in Salt Lake City more or less killed the hypothesis with a more rigorous investigation, showing in a Mormon population with good family histories that cystic-fibrosis carriers bore no more children than those without the mutation[18].

Jorde says that the best evidence for a heterozygous advantage has come from one of the mouse models. The bacterium that causes typhoid fever uses *CFTR* to enter cells, and mice heterozygous for the mutated gene seem to be resistant to infection[19]. If the same is true in humans, then heterozygotes would have had a selective advantage during typhoid epidemics. "Typhoid has been around a long time and killed a lot of people. If humans had resistance it would be a strong selection factor," Jorde says. That selection pressure would no longer be so prevalent, however, as the disease is controlled by vaccination in many countries. **H.P.**

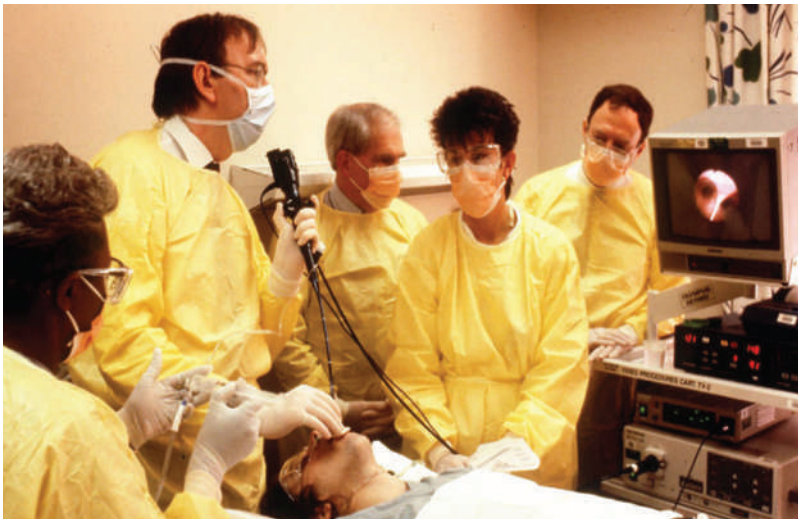
feature in his 40–50 pill-per-day regime, and he anticipates more advances that might improve his lung function. "Yes, we all hope for a cure, but if they can just help us stay healthy that in itself is quite an accomplishment," Bessette says.

Quinton, too, follows a rigorous regimen, inhaling hypertonic saline every day and taking intravenous antibiotics every few months. He rides his bike to work, but he can't run far or play basketball. Both upper lobes of his lung have been removed because of chronic inflammation. For someone born when he was, though, things could have been much worse — and thanks to research into the *CFTR* gene, Quinton knows why they're not. Although he has one copy of ΔF508, the mutation in his other gene, R17H, has relatively mild effects. He found this out when, several years after the gene was found, Garry Cutting at Johns Hopkins University School of Medicine in Baltimore, Maryland, analysed his genes as part of work on genetic testing for the disease.

These tests are "probably the most common form of genetic testing in the world today", says Cutting, who now drafts clinical-testing guidelines for cystic fibrosis. In the United States and some European countries many pregnant women and their partners are offered testing for mutations in *CFTR*, forcing clinical geneticists to confront issues about genetic counselling and genetic risk that are likely to escalate as more and more genes become as well studied. Working with a gene that has so many mutations, most of which are still little understood, underlines the futility of testing for something with no known clinical severity and therefore no rational basis on which to make decisions about ending a pregnancy. "The agony I've seen for some couples where one is a carrier and one has a mutation of unknown significance," Cutting says, "it is just immense." Newborn screening, which is also commonplace in some countries and typically involves a biochemical test followed by a genetic one, throws up similar issues for clinicians who may be unable to advise parents how severely their child is likely to be affected.

New studies are making the molecular landscape look even more complicated. Two years ago, the Cystic Fibrosis Foundation helped to launch a North American consortium to search for 'modifier genes' at work in the disease that might explain why some people with two copies of the ΔF508 mutation die at 16 whereas others have pretty healthy lungs into their 20s. The consortium members recently screened more than 4,500 people to look for genetic variations that are strongly linked with severity of the disease, says consortium member Michael Knowles from the University of North Carolina. One of the strongest variants to have emerged from previous studies of modifier genes, called TCF7L2, is also thought to strongly predispose carriers to type 2 diabetes[17]. The link may lie in the failure of the pancreas and consequent diabetes that cystic fibrosis frequently causes.

Results such as these suggest that once the *CFTR* gene and its protein are viewed in context, cystic fibrosis will spiral into a new realm of dizzying complexity. If studies of one gene have expanded to fill 20 years, how many years can be filled once the tens or even hundreds of modifier genes are factored in, let alone whatever other influences there may be outside the genetic code? For Knowles, though, the results present an exciting opportunity rather than a daunting complexity. He sees cystic fibrosis as "leading the way" for researchers investigating more genetically complex diseases. If he and others can get to grips with the numerous mutations in *CFTR* and its modifiers, they say that cystic fibrosis could serve as a case study for personalized medicine. Newborns identified with the disease could have their *CFTR* gene and other major modifier genes analysed to choose the most appropriate therapies — assuming, that is, that such a range exists by that point.



R. G. CRYSTAL/NIH

Early gene-therapy trials showed how difficult it is to express foreign genes in the lung.

"We have now tempered the optimism of the early '90s with a heavy dose of realism."
— Eric Alton

Although he has discovered molecular truths about himself that he might never have expected, "it would be hard for me to say I have benefited from the work I've done", Quinton says. Nonetheless, he, Riordan and others whose careers in this field stretch back farther than 1989 are still hopeful. Like most researchers and clinicians, they are focused on what they can achieve in the next 2–5 years, not what they should have achieved already. "I'd say don't give up," Quinton says. "This really is the only solution. As we succeed on one platform, it will make it much easier to succeed on another."

"It's a helluva lot more complicated than we realized," he says. "We went to the Moon in '69 and the conceit was we could do anything — we corrected polio, we wiped out smallpox. But when you start taking the system apart we've been really naive."

"But that's biology — it's not fair." ■

Helen Pearson is *Nature*'s chief features editor.

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