A New Approach to Cystic Fibrosis

Go after the defective gene, a group of researchers advised, using recent hints that a chloride channel defect causes the disease

Michael Brown of the University of Texas Health Sciences Center opened a recent meeting on cystic fibrosis with some consoling advice. "If you feel a little out of place at this meeting, don't worry," he told the participants. Brown and his colleague Joseph Goldstein had been charged by Claude Lenfant, director of National Heart, Lung, and Blood Institute, with bringing together about 100 scientists to discuss cystic fibrosis. They purposely included a majority whose work had *not* focused on this disease. Their reasoning, said Brown, was "to assemble a group of people including those who do clinical and fundamental research on cystic fibrosis together with basic scientists working on molecular areas that might be related in some way to cystic fibrosis and with a group of just bright, imaginative, creative people. Possibly, an idea might spring forth."

After 3 days, the group concluded that there is a need to try to find and clone the defective gene. In particular, the investigators were, with a few exceptions, ready to accept recent findings that cystic fibrosis might be linked to faulty chloride ion channels in cell membranes. "At least," said Goldstein, "that gives us a working hypothesis."

And a working hypothesis is sorely needed. The cystic fibrosis literature, says David Brock of Edinburgh University, "is voluminous and filled with contradictory and confusing accounts and unreproducible results. There are all kinds of observations that make one cry out, 'This can't be right.' "

Efraim Racker of Cornell University remarked, after surveying the cystic fibrosis literature, that, "anyone who isn't thoroughly confused just doesn't understand the situation." And Brock noted that the only statement that cystic fibrosis researchers could agree on at a workshop several years ago, was, "cystic fibrosis is inherited as an autosomal recessive disorder."

This is not an unusual situation. David Housmann of the Massachusetts Institute of Technology says that Huntington's disease research, for example, also was plagued by contradictory and unreproducible results. When a genetic disease affects many physiological systems and when the nature of the genetic defect is unknown, researchers frequently find secondary effects that they construe to be the primary malfunction.

Cystic fibrosis is the most common fatal genetic disease of Caucasians, and one for which there is no good treatment. One in 20 individuals is a carrier of the cystic fibrosis gene and the disease occurs whenever a child inherits two copies of the gene—one from each parent. It afflicts about 1 in 1800 whites.

The disease affects many different organ systems, but three of its manifestations are predominant. First, the patients have lung disease. As infants, cystic fibrosis patients get bacterial lung infections that persist throughout their lives. "Once the bacteria come in, we can't seem to kill them even with high doses of antibiotics," says Pamela Davis of Case Western Reserve University. Along with the lung infections, the patients secrete thick mucus that clogs the airways of their lungs. These two conditions lead to the breakdown of the walls of their airways. Eventually, most patients die of respiratory failure. In addition, the ducts of their pancreases get clogged by viscous secretions, leading to pancreatic insufficiency. This results in too few digestive enzymes in the gut, leaving the patients vulnerable to malnutrition. Finally, the patients have high concentrations of salt in their sweat—a condition used to diagnose the disease.

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Cystic fibrosis also can damage the liver through its thick mucus that clogs the liver's ducts, and, according to Davis, the few patients who do not die of lung disease die of cirrhosis of the liver and its complications.

Although they have only been able to offer supportive care and antibiotics to treat the frequent lung infections that plague cystic fibrosis patients, physicians have been able to greatly extend the life expectancy of these patients. As a result, Davis reports, the median age of survival has gone from less than 5 years in the late 1950's to about 21 years today. Yet, although most patients are reaching young adulthood, only a few survive through their 30's, according to Robert Beall of the Cystic Fibrosis Foundation in Rockville, Maryland.

The problem in cystic fibrosis research is how to tie it all together—the thick mucus, the effects on multiple organ systems, the salty sweat, and the increasingly convincing evidence from genetic studies that just a single gene is involved. What kind of gene could have these diverse effects and how can it be found? Recently, three groups of investigators have suggested that it might be a gene affecting chloride ion channels in cell membranes. The channels are the way that chloride enters and leaves cells—a process which seems to be completely passive, requiring no energy.

The most extensive evidence for a chloride channel defect was reported at the meeting by Paul Quinton of the University of California at Riverside. Quinton looked at sweat ducts, which he gets from small pieces of skin, each containing about four to eight sweat ducts. (People have about 2 to 5 million sweat ducts on their body.) Then he measures the passage of sodium and chloride through the membranes of the ducts by what he describes as a very simple experiment. He puts a duct in a solution of sodium chloride that is three times as concentrated as the sodium chloride inside the duct. Then, says Quinton, "we look at the way the sodium and chloride ions move across the membrane by measuring the potential. We get a current because the membrane is selective. Sodium and chloride both try to cross the membrane, and if the membrane is more permeable to one than the other, the solution inside the duct assumes the charge of the faster ion."

First, Quinton looked at ducts from normal individuals. The potential was −12 millivolts, which is quite small. Sodium is transported first by an energy requiring system and chloride follows by passing through a channel. The two ions both cross the membrane easily. In cystic fibrosis patients, the potential was −100 millivolts—exactly what would happen if the chloride channel was not functioning. Quinton recalls his amaze-

ment when he first saw this result. "I was watching the needle [measuring the potential] and it just kept going up. I couldn't believe it. It was the most glorious thing I have ever witnessed. If ever you have an experience in science, this is the sort you should have."

In addition to Quinton's finding, Richard Boucher of the University of North Carolina at Chapel Hill reported that the chloride flux in the nasal epithelia of cystic fibrosis patients is half that of controls. He removed nasal polyps from cystic fibrosis patients and cultured them in the laboratory before looking at how they transport chloride ions. As controls, he used polyps cut from the noses of hypoallergic hay-fever sufferers, who also are prone to develop these benign tissue growths.

The third piece of evidence implicating chloride channels in cystic fibrosis is from Jonathan Widdicombe of the University of California in San Francisco. He looked at tracheal cells from a person who had recently died from cystic fibrosis and found that their transport of chloride ions was blocked.

What is striking about the chloride channel results is that they were found in three different tissues known to be affected by the disease. And chloride channels are so very basic to cell functioning that blocked channels may cause multiple and disparate effects in different parts of the body.

The link between defective chloride channels and the thick mucus and respiratory disease that are so typical of cystic fibrosis is not clear. But, says Davis, "by all odds, it's the best lead we have. It is the only thing that connects the salty sweat with anything else." Davis notes that there are a number of possible connections between a chloride channel defect and the multiple effects of cystic fibrosis. For example, the respiratory disease could result from ion changes in the lungs that make them more vulnerable to infections. Or lung mucus could dry out because the water levels in airways are too low, due to a lack of chloride transport. That may prevent the epithelial cells of the lungs from being able to move mucus and foreign materials out of the airways. The result may be the buildup of thick mucus in the lungs.

But there is another problem with the chloride channel hypothesis. As one meeting participant pointed out, there is "the perennial question of heterozygotes." If the fundamental defect in cystic fibrosis really is faulty chloride channels, then heterozygotes, who carry one gene for the disease, might be expected to have a partial defect in the passage of chloride through their membranes. Quinton has not looked for a chloride channel defect in heterozygotes. Boucher has looked and sees nothing. And Widdicombe's research involves only a single patient—he too has not looked at heterozygotes.

There also are other possibilities. Amalia and Bronislaw Slomiany of New York Medical College, for example, think that the primary defect in cystic fibrosis is in an enzyme they recently discovered that puts fatty acids on glycoproteins. Cystic fibrosis patients have an overactive enzyme that adds nine to ten times more fatty acids than normal. Moreover, says Amalia Slomiany, "the abnormally viscous mucus of cystic fibrosis can be abolished by eliminating the excess fatty acids." And, she notes, "what we feel is that the enzyme we have discovered can modify glycoproteins destined for membranes as well. If so, it can modify chloride channels. We feel it is a primary modifier of tissues." The Slomianys have not yet looked at cystic fibrosis heterozygotes.

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But the basic sentiment at the meeting was to go for the simplest possible explanation of cystic fibrosis and that meant the chloride channel. Although there are various ways to get at the channel, Harvey Lodish of the Massachusetts Institute of Technology argues that it would not hurt to jump right in with the boldest experiment imaginable. Lodish and his colleagues Ronald Kopito and Seth Alper recently cloned a gene for "band 3," a major protein of red blood cell membranes that exchanges chloride for bicarbonate. Then, using a molecular probe for band 3 messenger RNA's, Lodish and his colleagues found that the gene is expressed in kidney cells and, intriguingly, that seemingly similar blood cell–derived genes are expressed in the kidney as well. Lodish speculates that these similar genes might be genes for chloride channels, and the band 3 gene might be part of a chloride channel gene family. If he is correct, his band 3 probe might also identify differences on the molecular level between cystic fibrosis cells and cells of normal individuals.

Lodish, acting on this hypothesis, approached Quinton at the meeting and said he wanted to send someone from his lab to Quinton's lab to look at sweat ducts with the band 3 probe. "How many sweat ducts?", Quinton asked. About 100 million, Lodish replied, meaning that he would need as many sweat ducts as are on the bodies of 20 people and would most likely have to work with the skin of cadavers. But Lodish is not deterred. If the experiment works, he will have demonstrated that the chloride channel genes of cystic fibrosis patients are not being properly expressed and, because he has a molecular probe, he would presumably be able to detect cystic fibrosis carriers and do prenatal diagnoses.

Another way to get at the fundamental defect in cystic fibrosis is to go the Huntington's disease route. About 1-1/2 years ago, a group of researchers found a marker that seems to lie close to the Huntington's disease gene and are now closing in on the gene itself. Once they identify the gene, they hope to be able to learn what it does and what goes wrong in Huntington's disease. In the meantime, they are able to tell many people at risk for Huntington's disease whether they have the gene and are able to do some prenatal diagnoses.

But finding a genetic marker requires having families that carry the gene and have large pedigrees. The idea is to find some restriction enzyme marker that is inherited along with the gene for the disease. What made the Huntington's disease gene study work was an enormous family in Venezuela that has carried the gene for more than a century. No such family is known for cystic fibrosis. Nonetheless, several groups of researchers are now working with what they have to try and find a cystic fibrosis gene marker. Among them is Housmann, whose company, Integrated Genetics, is looking for a marker among an Amish family whose pedigree is known for nine generations. Housmann is a member of the group that found the Huntington's disease gene marker.

At the close of the meeting, if an idea could be said to spring forth it was to change the focus of cystic fibrosis research from one of searching for descriptions of what, biochemically, could be wrong, to working with the powerful tools of molecular biology to find the actual nature of the gene defect, if not the gene itself. The group was split on whether to go for the gene marker or the defective protein but, as Brown remarked, "nothing should be ruled out."—GINA KOLATA