



**JOHNS HOPKINS**  
M E D I C I N E

News from the Cystic Fibrosis Center  
at Johns Hopkins

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# Partners IN DISCOVERY

Translating Theory into Therapy

## Research Briefs

### IMPORTANT GENE THERAPY TRIAL ENROLLS ITS LAST PATIENT

The fifteenth and final patient enrolled at Johns Hopkins in November, 2004 was also the hundredth and final patient enrolled nationwide in a multi-center trial of adeno-associated virus (AAV) gene therapy. This trial—the largest gene therapy trial ever conducted—is critical because it was designed to confirm previous research that showed AAV vectors carrying a normal CFTR gene could actually improve lung function (*Chest*, February 2004, volume 125, pages 509-21). The current study will test the effect of giving two doses of the same AAV vector to 50 CF patients compared to those who receive a placebo. The results should be available later this year.

This moves us toward effective gene therapy for CF. However, the best system to deliver a normal copy of the CFTR gene has not yet been determined. AAV vectors were created by a partnership between Johns Hopkins, the National Institutes of Health and Targeted Genetics, a biotechnology company in Seattle, WA. Positive results from this trial will ensure that AAV development will continue. Trials of a newer AAV type 5 vector (*Partners in Discovery*, August 2004) will begin at Johns Hopkins in the fall.

### CURCUMIN STUDY TO START SOON

Curcumin, a component of the Indian spice turmeric, has been shown to improve survival of mice with CF. A Phase I safety study of this spice in adult CF patients will begin in the spring at Johns Hopkins and the University of Washington. Approximately five patients at each site will be treated with curcumin for two weeks. Although this is a safety study, the effect of curcumin on lung function and nasal potential difference will also be

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## Environment, More Than Genes, Found to Most Influence CF Lung Disease Severity



Geneticist Garry Cutting

As scientists continue to learn more about the gene responsible for cystic fibrosis—CFTR—it is becoming clear that this genetic mutation alone does not determine why the severity of CF differs so much from person to person. Why does one patient only have mild lung disease while another patient, with the same CFTR mutations, is debilitated with severe lung disease? Are other genes responsible for these variations or is the environment the cause?

In an attempt to answer these questions, researchers at Johns Hopkins launched an unprecedented nationwide study focusing on twins with CF—an ideal group for studying CF disease variation, according to geneticist Garry Cutting, M.D., because twins share genetic makeups and similar environments. To help refine this search for modifying genes,

the study was later enlarged to include siblings with CF.

The CF Twin and Sibling study, currently in its fourth year, will ultimately create a comprehensive clinical database and DNA collection on twins and siblings with CF that will enable researchers to look for environment and genetic factors that modify the course of CF and its symptoms.

Early results are surprising: environment—not modifier genes, as originally suspected—has a major influence upon the severity of CF lung disease. Researchers found that identical twins with CF have remarkably similar degrees of lung disease when living together compared to those who live apart. Because identical twins share the same exact genetic makeup, the only variable that exists is their environment.

“When identical twins live together, they

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## NACFC Highlights

Below are highlights of the 18th North American Cystic Fibrosis Conference (NACFC), held in St. Louis, Missouri October 14-18, 2004.

### HIGH THROUGHPUT SCREENING SUCCESSES

Two research groups reported on progress made by the CF Foundation's high throughput screening program in a session chaired by Pam Zeitlin, M.D., Ph.D., of Johns Hopkins. Both groups are trying to identify compounds called potentiators that improve chloride transport through  $\Delta F508$ -CFTR, and compounds called correctors that allow more  $\Delta F508$ -CFTR to reach the cell surface. The effective compounds are identified by testing hundreds of thousands of chemicals on CF cells. The promising “hits” can then be studied in more detail and altered to optimize their function and minimize any toxicity, thus creating “leads” for further research.

Alan Verkman, Ph.D., from the University of California at San Francisco described his laboratory's work in identifying several classes of chemicals that are either potentiators or correctors. Further modification of the most promising “leads” is now underway. Verkman's laboratory has also discovered a potent inhibitor of CFTR. This is an exciting find as it could be used to create a new animal model of CF that develops lung disease for future study. Paul Negulescu, Ph.D., from Vertex Pharmaceuticals spoke about his company's discovery of four potentiators and two correctors. These “hits” are also being aggressively studied and optimized.

### RATIONAL DRUG DESIGN

In order to develop new drugs to treat CF, researchers need to understand the function of the cystic fibrosis transmembrane conductance regulator (CFTR) protein. One impor-

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## A Cure is on the Way



... but in the meantime, preserving lung function is crucial. Everyone's lung function declines as they get older but the decline is faster in individuals with CF. Airway

clearance, preventative antibiotics and adequate nutrition are all designed to keep CF lungs healthy. In fact, the goal of all our current therapies is to slow the rate of lung function decline.

Why am I writing about these rather mundane "older" treatments when there are so many exciting therapies on the horizon? First and foremost ... slowing the decline of lung function will improve survival. Additionally, when a cure comes it will not reverse lung damage that has already occurred. New therapies are going to build on the foundation that is present in each individual's lungs. That is why early intervention in young children with CF is so important. But that is only part of the answer. Patients with CF must do everything possible to keep their lungs as healthy as they can be at every age.

The ongoing search for new therapies is yielding exciting results. The projects discussed in this issue of *Partners in Discovery* will not cure CF but they may slow the loss of lung function and improve survival not by years but by DECADES. However, until these treatments are on the market, consistent application of the tried and true older therapies is vitally important.

Peter J. Mogayzel Jr., M.D., Ph.D.  
Director, Cystic Fibrosis Center  
at Johns Hopkins

## Geneticist Garry Cutting, M.D.

**Although cystic fibrosis is caused by mutations in the gene that makes a protein called CFTR (cystic fibrosis transmembrane conductance regulator), a broad spectrum of disease severity exists. Some patients with CF die early in childhood while others live well into adulthood with only mild lung or liver disease. Evidence is accumulating that shows the CFTR genotype alone does not account for this wide diversity. Rather, it is the interaction of the environment and other non-CFTR "modifier" genes that determines the clinical course of CF.**

### What are gene modifiers?

In terms of cystic fibrosis, modifier genes are genes other than the CF gene (CFTR) that may have an effect on how the body responds to the conditions that develop as the result of defective CFTR.

### Have any gene modifiers been identified?

Researchers have studied a number of potential CF gene modifiers; however, none have been clearly substantiated. Potential modifiers of CF are chosen to reflect the known pathophysiology of CF, including genes involved in the patient's response to infection and CF complications such as intestinal obstruction, diabetes, and liver disease. The general classes of potential modifier genes include inflammatory and anti-inflammatory

mediators, antioxidants, mediators of airway reactivity, molecules involved in CFTR trafficking, and alternative ion channels. The best-studied CF candidate modifiers include mannose-binding lectin (MBL), glutathione-S-transferase, transforming growth factor-beta1 (TGF-β1), tumor necrosis factor-α (TNF-α), adrenergic receptor, and HLA class II antigens.

In a preliminary study, Johns Hopkins researchers have identified two genes associated with CF survival—MBL and TNF-α—although these findings have yet to be confirmed by other studies. If confirmed, researchers say it may be possible to type patients for these genes in order to provide a better idea of outcome. Targeted gene therapies could also be developed. ■



## Environment Influences Disease

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share the same environment—be it living conditions within the home, breathing in the same air, eating the same diet, and going to the doctor at the same time," Cutting says. "In this study, we've seen twins who move apart and suddenly one twin develops severe lung disease while the other remains mildly affected. Because identical twins do not differ genetically, we can only assume that something in the environment is responsible for the difference in lung disease severity."

Cutting says the discovery that environment plays a significant role in CF lung disease is good news for patients, adding that "it's easier to change

things in our environment than it is to change our genes."

A new joint study with the Johns Hopkins Bloomberg School of Public Health will focus on the environments of identical twins with CF who live together and apart. Through home visits to these twins, researchers hope to identify what it is about their environments that both positively and negatively affects lung disease. "With a better understanding of these environmental factors, we can provide CF patients and their families with the tools to better protect themselves from lung disease," Cutting says. ■

### CF Research Web sites at Johns Hopkins

CF Research and Development Center:  
[www.johnshopkinscfresearch.org](http://www.johnshopkinscfresearch.org)  
CF Genotyping Center:  
[www.hopkinsmedicine.org/cfgenotyping](http://www.hopkinsmedicine.org/cfgenotyping)  
CF Microarray Center:  
[www.hopkins-genomics.org](http://www.hopkins-genomics.org)

# Surgeon Anne Fischer: A Quick Study on CF Gene Therapy Models



Anne Fischer

**F**or pediatric surgeon Anne Fischer, M.D., Ph.D., there were interests beyond the scalpel in coming to the Johns Hopkins Children's Center in 1999. With training also as an immunologist, she wanted to study gene therapies for a serious disease in the hopes of preventing or halting it before surgery was a patient's only option. Cystic fibrosis, afflicting about 30,000 children and adults in the United States, was certainly a candidate. And Johns Hopkins, with its large-scale CF Center, was certainly a venue to take it on.

"Not many places treat 400-plus patients

**"Here's something—a monogenetic, not a polygenetic disease—in which gene therapy was potentially very doable and could have a huge impact."**

every year," Fischer says, "nor have this overlap of scientists, researchers and clinicians from multiple disciplines studying it."

Fischer says she saw CF as sort of a poster child for gene therapy, in that only one highly studied gene, the CFTR gene, has been found to be at play in the disease. Discovered in 1989, that gene writes the instructions for the protein cystic fibrosis transmembrane conduc-

tance regulator, a channel that crosses cell membranes.

More than 1,100 mutations in CFTR have been found to cause CF by blocking the movement of salt and water across this channel into cells in tissues that produce mucus, saliva and digestive enzymes. Consequently, cells that line the passageways of the lungs, pancreas and other organs produce the abnormally thick, sticky mucus characteristic of CF. But, if you could deliver a healthy gene to override the defective CFTR gene, you could potentially stop the hyperviscosity of the mucus, the subsequent bacterial infections and the permanent lung damage they lead to.

"Here's something—a monogenetic, not a polygenetic disease—in which gene therapy was potentially very doable and could have a huge impact," Fischer says.

Fischer explains that Hopkins researchers, led by the collaborative efforts of William Guggino, Ph.D., and pediatric pulmonologist Pamela Zeitlin, M.D., Ph.D., who have been studying CF gene therapies since 1994, have shown that the adeno-associated virus, or AAV, is an effective, safe vehicle for delivering normal genes. In using repeated doses of an inhaled aerosol solution, Zeitlin found AAV2 had minimal inflammation and improved lung function with no adverse effects (*Chest*, 2004, Volume 125, pages 509–521).

But there was one problem, and not a small one. While transfer of the healthy genes was

detected, it was unclear whether they maximally produced normal proteins—a process known as gene expression. This find opened the door for Fischer, who has been searching for other AAV serotypes that have stronger gene expression in CF. Then, in animal models, Fischer and others found that AAV5 more readily binds to the airway surface and epithelia. The result? A 20- to 50-fold increase in DNA expression (*Partners in Discovery*, August 2004). More successful gene transfer should translate into greater gene expression.

"It's a serotype that has higher propensity, the receptors are right on the respiratory surface and there're more of them," says Fischer. "An airway epithelium wants to take up AAV5 much more than it does AAV2."

Fischer continues to investigate other serotypes in the AAV family to see if they may enhance DNA transfer even more. The implication, among others, is a less viscous mucus.

"If we can keep the hyperviscosity and infections at bay," Fischer explains, "then patients will live even longer."

Coming full circle, Fischer concedes she has a surgical interest in her research, as well. If clinician-scientists like her can come up with viable gene transfer models, then surgeons like her may be able to deliver them via fetal surgical interventions prior to any evidence of disease. "As a pediatric surgeon," she notes, "I'm the perfect person for that." ■

## Family Education Day

More than 100 people enjoyed informative seminars and socializing at CF Family Education Day, November 13, 2004, at Johns Hopkins. Some highlights:

In recent years the CF community has become aware of the potential for bacteria to be transmitted from one CF patient to another. Guest speaker Lisa Saiman, M.D., an infectious disease expert from Columbia University and Children's Hospital of New York, described studies that demonstrated this bacterial transmission and reviewed potential methods to prevent it. Saiman chaired the committee that formulated the CF Foundation guidelines for infection control. The Johns Hopkins CF Center has instituted policies that follow the CF Foundation guidelines.

Michael Boyle, M.D., director of the adult CF program at Johns Hopkins, discussed reproductive issues for CF patients. He pointed out that we used to ask, "Can CF patients have children?" The answer is YES. However, it's more important for patients to ask themselves, "Is having children right for me?" Improving survival has made having a family a reality rather than a dream for patients with CF. Pamela Zeitlin, M.D., Ph.D., reviewed current research at Hopkins. CF Center physical therapists Shelly Trivett and Karen Hussey gave an overview of airway clearance techniques. A parent panel discussed nutritional issues that confront children with CF. The day ended with small focus groups discussing a range of topics. ■

## Research Briefs

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measured. If this study shows curcumin to be safe, a larger study may be undertaken later in the year to determine if this spice actually improves CF lung disease.

### FOLLOW-UP INS37217 STUDY PLANNED

The exciting finding that inhaled INS37217 improved lung function in a recent Phase II safety study prompted Inspire Pharmaceuticals to plan a larger trial later this year. Although few details are currently available, this multi-center trial will continue the path of this drug toward a large Phase III trial involving hundreds of CF patients and an eventual application for Food and Drug Administration approval.

### DO YOU KNOW YOUR CFTR MUTATION?

Over 1,100 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene have been discovered to date. A person must have inherited two copies of a mutation—one from each parent—to have CF. Many studies focus on patients with two  $\Delta F508$  mutations because this is most common. However, studies are underway and specific therapies are being developed for CF patients with other mutations, such as stop codon mutations (those that end in X such as W1282X). Several patients at Johns Hopkins participated in a study of gentamicin last year. In addition, the search for modifier genes that affect CF lung disease has now been expanded to include patients with other CFTR mutations. A blood sample is needed for this study which can be obtained along with your annual blood tests.

Even after testing for most known CFTR mutations, the cause of some patients' CF remains unknown. Peter Mogayzel, M.D., Ph.D., and Garry Cutting, M.D., are studying these patients to determine if other rare mutations are present that shorten the CFTR protein or if a mutation exists outside the CFTR gene that could affect how much protein is produced. ■

# What is early intervention?

**S**lowing the progression of lung disease is the key to longer survival for patients with CF. Today, physicians are focusing more attention on infants and young children with CF to intervene before significant lung disease develops. This “early intervention” uses more preventative therapies, and treats problems in children with mild CF lung disease more aggressively. The ongoing study at Johns Hopkins measuring lung function in young children with infant pulmonary function testing is a good example.

We've known for some time that children who acquire the bacteria *Pseudomonas aeruginosa* at a younger age tend to have a faster decline in lung function. Recent research in European CF centers has suggested that treatment with antibiotics when pseudomonas is first detected could delay colonization with this bacterium. These findings have led to earlier therapy when pseudomonas is first detected.

At Johns Hopkins, we've been treating children with newly acquired pseudomonas with a combination of inhaled and oral antibiotics for the past two years. However, the optimal therapy for these children remains unclear. To determine the best early intervention for newly acquired pseudomonas, the Early Pseudomonas Intervention Control (EPIC) trial will begin this spring, involving 1,400 patients at more than 50 CF centers across the US. Children with pseudomonas will be treated with one of four combinations of oral or inhaled antibiotics for up to 18 months. Peter Mogayzel, M.D., Ph.D., will direct this clinical trial at Johns Hopkins. ■

## NACFC Highlights

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tant clue to understanding function is to look at a protein's structure—a daunting task for CFTR because it sits in the cell membrane and is therefore difficult to purify for study. Two groups made great progress this year in this area. John Riordan, M.D., a co-discoverer of the CFTR gene, has published the first pictures of CFTR in the September 2004 issue of the

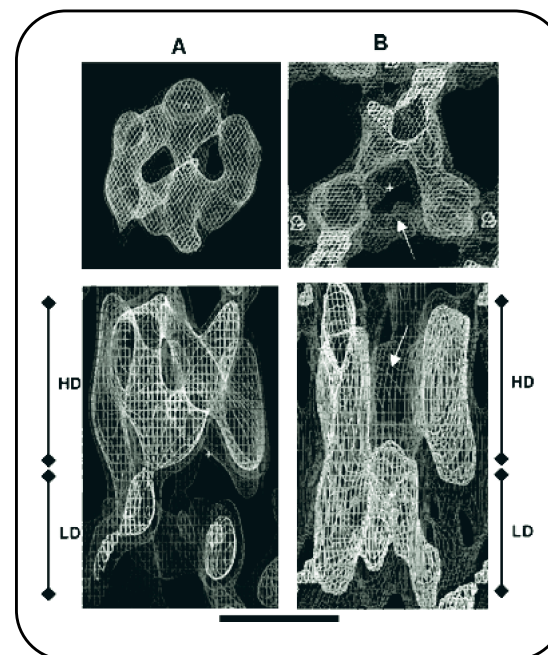
researchers create drugs targeting specific regions of CFTR.

### HOW MUCH CFTR IS ENOUGH?

Now that researchers have identified several exciting drug and genetic therapies that could improve defective CFTR function, we're asking a vital question: How much CFTR function

is needed to prevent CF lung disease? Several sessions at this year's NACFC were devoted to this topic. Clearly, reducing CFTR function by 50 percent does not lead to lung disease since CF carriers (parents of children with CF) do not have lung disease. We also know that patients with a related CFTR disorder called congenital bilateral absence of the vas deferens, or CABVD, do not

have lung disease, and they have between 10 to 20 percent of normal CFTR function. Patients with mild CF lung disease may have as little as 5 percent of normal CFTR function. Therefore, new therapies may only have to restore 5 to 10 percent of normal CFTR function to prevent severe lung disease. Several studies are underway across the country to help us determine the degree of CFTR function necessary to preserve lung function. ■



*Journal of Biological Chemistry* (volume 279, pages 39051-39057). The figure above shows the pore that transports chloride through the cell membrane. Although this is considered a “low resolution” view of CFTR, it is a significant milestone. The San Diego biotechnology company Structural GenomiX, now SGX, has discovered the structure of the region of CFTR that contains the  $\Delta F508$  mutation. This new information will help



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