What Does CFTR Tell Us About Lung Disease?

In this Issue...

In 1989 the gene that causes cystic fibrosis (CF) was identified, an event many look at as the beginning of modern molecular medicine. The advances continue, as our understanding of the CF transmembrane conductance regulator (CFTR) and the mutations that cause CF become more and more important for providers who treat CF to integrate into their practices.

In this issue, we review published research:

- studying the first large series of patients taking the drug ivacaftor, the first example of an FDA and EU approved therapy that targets the consequence of a specific CFTR mutation (G551D)
- examining the use of ivacaftor in individuals with the F508del mutation
- describing how CFTR genotype may be helpful in predicting prognosis, how genotype may be used diagnostically, and how sometimes CFTR mutation can result in a disease other than CF
- updating the CFTR2 database, where frequently seen CFTR mutations were analyzed for clinical, functional, and population parameters to define their severity

LEARNING OBJECTIVES

After participating in this activity, the participant will demonstrate the ability to:

- Recognize that ivacaftor is a new therapy that has been shown to correct the CFTR protein and improve lung function in patients with CF who have the G551D mutation.

- Describe how the CFTR genotype influences lung function and understand factors accounting for variability in lung function, even among people with the same genotype.

- Describe the use of CFTR genotype to facilitate making diagnoses of CF, predicting prognoses, and selecting therapies.

The Johns Hopkins University School of Medicine takes responsibility for the content, quality, and scientific integrity of this CME activity.
The Institute for Johns Hopkins Nursing and the American Nurses Credentialing Center do not endorse the use of any commercial products discussed or displayed in conjunction with this educational activity.

CREDIT DESIGNATIONS
Physicians
Newsletter: The Johns Hopkins University School of Medicine designates this enduring material for a maximum of 1.0 AMA PRA Category 1 Credit(s)™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Nurses
Newsletter: This 1 contact hour Educational Activity is provided by the Institute for Johns Hopkins Nursing. Each Newsletter carries a maximum of 1 contact hours or a total of 6 contact hours for the six newsletters in this program.

Respiratory Therapists
For United States: Visit this page to confirm that your state will accept the CE Credits gained through this program.

For Canada: Visit this page to confirm that your province will accept the CE Credits gained through this program.

INTENDED AUDIENCE
This activity has been developed for pulmonologists, pediatric pulmonologists, gastroenterologists, pediatricians, infectious disease specialists, respiratory therapists, dieticians, nutritionists, nurses, and physical therapists.

There are no fees or prerequisites for this activity.

LAUNCH DATE
This program launched on February 28, 2013 and is published monthly; activities expire two years from the date of publication.

HARDWARE & SOFTWARE REQUIREMENTS
Pentium 800 processor or greater, Windows 98/NT/2000/XP or Mac OS 9.x, Microsoft Internet Explorer 5.5 or later, Windows Media Player 9.0 or later, 128 MB of RAM Monitor settings: High color at 800 x 600 pixels, Sound card and speakers, Adobe Acrobat Reader.

DISCLAIMER STATEMENT
The opinions and recommendations expressed by faculty and other experts whose input is included in this program are their own. This enduring material is produced for educational purposes only. Use of Johns Hopkins University School of Medicine name implies review of educational format design and approach. Please review the complete prescribing information of specific drugs or combination of drugs, including indications, contraindications, warnings and adverse effects before administering pharmacologic therapy to patients.

STATEMENT OF NEED
Based on a review of the current literature, including national and regional measures, detailed conversations with expert educators at Johns Hopkins, and a survey of potential program participants, this program will address the following core patient care gaps:

- **Pulmonary Infections**: Many clinicians lack strategies to persuade patients to accept ABX to treat chronic pulmonary infections.
- **Genotype and Therapy**: Clinicians may be uncertain how to integrate introduced disease-modifying therapies and how they are altering the therapeutic landscape for patients with cystic fibrosis.
- **Drug Therapy**: Clinicians may be uncertain how to integrate genotyping into therapeutic decisions and how to communicate with patients and families about the relationship between genotype and therapy.
- **Weight Gain**: Many clinicians lack strategies to persuade patients to adhere to CF nutritional requirements, resulting in low body weight and nutritional failure in patients with cystic fibrosis.
- **Pancreatic Function**: Many clinicians remain uncertain how to optimize pancreatic function in patients with cystic fibrosis.

**TREATING CF PATIENTS WITH INHALED ANTIBIOTICS**

- Many clinicians lack knowledge about the use of existing and emerging inhaled ABX to treat chronic pulmonary infections.

Michael P. Boyle, MD, FCCP discloses that he has served as a consultant for Vertex, Novartis, Genentech, Savara, Pharmaxis, and Gilead Sciences, Inc. He has also received grant/research support from Vertex.

No other planners have indicated that they have any financial interests or relationships with a commercial entity.

Guest Author's Disclosures
This activity is supported by educational grants from Aptalis Pharma, Gilead Sciences, Inc, and Vertex Pharmaceuticals.

SUCCESSFUL COMPLETION
To successfully complete this activity, participants must read the content, and visit the Johns Hopkins University School of Medicine's CME website and the Institute for Johns Hopkins Nursing. If you have already registered for other Hopkins CE programs at these sites, simply enter the requested information when prompted. Otherwise, complete the registration form to begin the testing process. A passing grade of 70% or higher on the post-test/evaluation is required to receive CE credit.

STATEMENT OF RESPONSIBILITY
The Johns Hopkins University School of Medicine takes responsibility for the content, quality and scientific integrity of this CME activity.

CONFIDENTIALITY DISCLAIMER FOR CME CONFERENCE ATTENDEES
I certify that I am attending a Johns Hopkins University School of Medicine CME activity for accredited training and/or educational purposes.

I understand that while I am attending in this capacity, I may be exposed to "protected health information," as that term is defined and used in Hopkins policies and in the federal HIPAA privacy regulations (the "Privacy Regulations"). Protected health information is information about a person's health or treatment that identifies the person.

I pledge and agree to use and disclose any of this protected health information only for the training and/or educational purposes of my visit and to keep the information confidential.

I understand that I may direct to the Johns Hopkins Privacy Officer any questions I have about my obligations under this Confidentiality Pledge or under any of the Hopkins policies and procedures and applicable laws and regulations related to confidentiality. The contact information is: Johns Hopkins Privacy Officer, telephone: 410-735-6509, e-mail: HIPAA@jhmi.edu.

"The Office of Continuing Medical Education at the Johns Hopkins University School of Medicine, as provider of this activity, has relayed information with the CME attendees/participants and certifies that the visitor is attending for training, education and/or observation purposes only.

For CME Questions, please contact the CME Office at (410) 955-2959 or e-mail cmenet@jhmi.edu.

For CME Certificates, please call (410) 502-9634.

Johns Hopkins University School of Medicine
Office of Continuing Medical Education
Turner 20/720 Rutland Avenue
Baltimore, Maryland 21205-2195

Reviewed & Approved by:
General Counsel, Johns Hopkins Medicine (4/1/03)
Updated 4/09

INTERNET CME/CE POLICY
The Office of Continuing Medical Education (CME) at the Johns Hopkins University School of Medicine is committed to protecting the privacy of its members and customers. The Johns Hopkins University SOM CME maintains its Internet site as an information resource and service for physicians, other health professionals and the public.

Continuing Medical Education at the Johns Hopkins University School of Medicine will keep your personal...
Clinicians need more information to make informed decisions about the use of inhaled ABX in combination.

Clinicians lack information about best practices for scheduling ABX therapy to suppress chronic airway infections.

Common clinician assumptions about treating pulmonary exacerbations lack supporting evidence.

CF clinicians are not aware of and/or are not actively advocating inhaled ABX patient-adherence strategies.

and credit information confidential when you participate in a CME Internet-based program. Your information will never be given to anyone outside the Johns Hopkins University School of Medicine's CME program; CME collects only the information necessary to provide you with the services that you request.

IN THIS ISSUE

- **Commentary**
- **Ivacaftor for individuals with CF due to at least one G551D CFTR mutation**
- **Ivacaftor in individuals with CF and the F508del CFTR mutation**
- **CFTR Genotype and Prognosis**
- **Defining CFTR-related disorders**
- **The Clinical and Functional Translation of CFTR project (CFTR2)**

Planning Committee

Michael P. Boyle, MD, FCCP
Associate Professor of Medicine
Director, Adult Cystic Fibrosis Program
The Johns Hopkins University
Baltimore, MD

Peter J. Mogayzel, Jr., MD, PhD
Professor of Pediatrics
Director, Cystic Fibrosis Center
The Johns Hopkins University
Baltimore, MD

Donna W. Peeler, RN, BSN
Pediatric Clinical Coordinator
Cystic Fibrosis Center
The Johns Hopkins University
Baltimore, MD

Meghan Ramsay, MS, CRNP
Adult Clinical Coordinator
Cystic Fibrosis Center
The Johns Hopkins University
Baltimore, MD

GUEST AUTHOR OF THE MONTH

Commentary & Reviews:

Patrick Sosnay, MD
Assistant Professor of Medicine
Division of Pulmonary & Critical Care Medicine,
McKusick-Nathans Institute for Genetic Medicine
Johns Hopkins Cystic Fibrosis Center
The Johns Hopkins University
Baltimore, MD

Guest Faculty Disclosures

The author has indicated that he does not have financial interests or relationships with a commercial entity.

Unlabeled/Unapproved Uses

The author has indicated that there will be no reference to unlabeled or unapproved uses of drugs or products.

Planning Committee Disclosures

COMMENTARY

Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which forms a protein that transports chloride and helps cells maintain a constant external environment. Shortly after the gene was identified, the CF Mutation Database was established to catalog all variants in the gene ([www.genet.sickkids.on.ca](http://www.genet.sickkids.on.ca)). Currently there are almost 2000 different variants described on this website. Some have been identified in individuals with CF, but only a few variants show clear evidence that they either cause CF or represent a genetic difference that does not cause disease. Several innovations require that we have a greater understanding of the genetic epidemiology of CF, the mechanism by which the mutations disrupt CFTR function,
and the range of phenotypes displayed in patients who carry a mutation. This newsletter issue and next month's podcast will illustrate how the genetics of CF can be used to make a diagnosis or identify a CF carrier, predict how individuals with a given mutation will do clinically and allow clinicians to select currently available and hopefully future therapies that directly address the defect caused by their individual CFTR mutations.

Cystic Fibrosis is an autosomal recessive disorder; CFTR genes carrying CF-causing mutations must be present in both parents and passed on to the offspring to result in a baby with CF. A single CFTR copy is sometimes referred to as an allele. The two alleles that we all possess are called a CFTR genotype. The terms mutation and variant are commonly used to describe changes in a gene that make it different from the widely recognized most common version of the gene (geneticists refer to this as the "wild-type" version). It is important to note that not all differences from wild-type CFTR cause CF. Some genetic variations have no effect on protein function, others cause the protein to be completely disabled or not produced at all, while others fall somewhere in between. In general, there is good evidence that the more severely a variation disrupts or disables CFTR, the more likely patients carrying a copy of that mutation on both of their CFTR gene copies will have the disease CF.

Genetic variants can be labeled for the change they produce in the protein (F508del, G551D, or W1282X are all examples of this) or for the change they cause in the nucleotides (the DNA base pair sequence, for example 1717-1G->A or 3849+10kbC->T). A few uncommon mutations are named for changes they produce in the exons, pieces of DNA that will eventually be assembled into a mature protein (an example of this is CFTRdel2,3). As part of a movement in the genetics fields, some mutation names that CF researchers have used since the gene was found may be reported differently on diagnostic lab reports or in scientific studies — for example, F508del can be referred to as p.Phe508del or as c.1521_1523delCTT). The CF mutation database website (www.genet.sickkids.on.ca) and the CFTR2 site (www.cfr2.org) can be used to search between the legacy (traditional) and new nomenclature.

Cystic Fibrosis mutation analysis is commonly used to aid in the diagnosis of CF and in screening for carriers. The most recent guidelines on CF diagnosis require that a patient have both clinical symptoms (which could be the typical organ system manifestations of CF disease, or a newborn test for the immunoreactive trypsinogen, or a sibling with CF), and evidence of CFTR dysfunction. The criteria of CFTR dysfunction can be either an elevated sweat chloride or the presence of two CF-causing mutations. In carriers who do not have symptoms as well as in newborns who may not yet have developed organ system disease, CFTR genetic analysis plays a greater role. At this time, genetic testing technologies have become less expensive and more widely available. Increasingly, the technique of sequencing the gene and looking at each nucleotide is replacing panel testing that looks only for specific mutations. The advantage of sequencing is that it can identify infrequently seen mutations that may not be contained in a panel of common CFTR mutations; however, the consequence is that variants with uncertain disease consequence may be identified.

A more challenging way to correlate CFTR genotype with phenotype extends beyond using CFTR genotype to diagnose CF toward using CFTR genotype to predict the disease course in a person with CF. Scientists have identified several ways that CFTR mutations could disrupt the CFTR protein. It was recognized that classes of mutation that retained some residual CFTR function were more likely to be associated with pancreatic-sufficient CF. Lung function, which is most important to CF patients' morbidity and mortality, has a less clear correlation between genotype and phenotype. Lung function varies tremendously, even among patients with the same genotype. That variability is related to other genes as well as to environmental factors. The variability and lack of clear genotype-phenotype correlation for lung disease means that for any given individual, there is no reliable way to predict lung function over time.

Since the identification of CFTR, the CF field has searched for ways to directly correct the defects caused by CFTR mutation. The first therapy to improve CFTR function is ivacaftor, which is effective for individuals with at least one G551D CFTR mutation, is discussed in this newsletter. Experimental evidence shows that ivacaftor may also benefit other mutations that affect CFTR gating. The concept of classes of CFTR mutations will hopefully be replaced with mutation "theratypes" that are grouped by their response to a given type of drug rather than molecular defect. As we move toward this era, the priority will be finding therapies for the mutations that affect the most patients. It makes sense to be able to try these therapies in as many mutations as possible. For that reason, there is a greater need to understand the mechanism of all CFTR mutations, even the rarely seen ones.
Throughout medicine there is a shift toward tailoring therapies to an individual patient. Even in a Mendelian disease such as CF, this personalized approach is appropriate. Great strides have been made in CF care in the last several decades, with an encouraging increase in life expectancy. The improvements in airway clearance/maintenance, nutrition, and antimicrobials, as well as centralized care and attention to adherence, have all had great benefit. Bringing about a dramatic change will require a new type of therapy that alleviates defective CFTR function and can be given safely for a long time. This individualized approach to therapy will require the mutations that contribute to the differences in our CF patients to be well characterized.

**Commentary References**


---

**IVACAFTOR FOR INDIVIDUALS WITH CF DUE TO AT LEAST ONE G551D CFTR MUTATION**


*(For non-subscribers to this journal, an additional fee may apply to obtain full-text articles.)*

**View journal abstract**  **View full article**

This study, published by Bonnie Ramsey from the University of Washington along with an international team of CF clinicians and researchers and funded by the US Cystic Fibrosis Foundation and Vertex pharmaceuticals (the manufacturer of the drug), led to the approval of the oral drug ivacaftor by the US Food and Drug Administration (FDA). Following up on a promising Phase II trial on the drug published a year earlier, the authors randomized 168 patients to receive either ivacaftor (150 mg twice daily) or placebo for 48 weeks. Study participants had at least one copy of the G551D mutation, be older than age 12, and have a lung function (FEV$_1$ percent predicted) between 40%-90% of normal.

Patients receiving ivacaftor showed improvement in lung function within two weeks which was sustained throughout the trial (absolute improvement in FEV$_1$ vs. placebo differed at each time point but was approximately 10% and sustained; $P < 0.001$ at each time point). Lung function at 24 weeks was the primary endpoint of the trial, but many other secondary endpoints favored ivacaftor treatment as well. The ivacaftor group experienced 55% fewer pulmonary exacerbations compared to the control group, with 47 exacerbations (in 21 subjects) in the ivacaftor group compared to 99 exacerbations (in 44 subjects) in the group that received placebo ($P = 0.001$ for the reduction in hazard ratio). Participants completed a questionnaire that assessed respiratory symptoms and quality of life; the ivacaftor group showed improvement and the placebo group had a slight decline over the 48 weeks of the trial ($P < 0.0001$). The ivacaftor-treated patients gained on average 3.1 kg (6.8 lbs.) vs an average weight gain in the placebo group of 0.4 kg (0.9 lbs.) ($P < 0.0001$).
Consistent with earlier trials, there was evidence that ivacaftor was working to restore CFTR function. The commonly performed diagnostic test for CF, the measurement of sweat chloride concentration, assesses CFTR activity of reabsorbing chloride from the sweat glands. A lower sweat chloride level correlates with increased activity of CFTR. Remarkably, the patients on ivacaftor had a decrease in their sweat chloride concentration that was 44 mmol/L lower than placebo treated patients (P < 0.0001). This decrease meant that the ivacaftor-treated patients had a mean sweat chloride that was 47 mmol/L, below the cut-off for definitive CF diagnosis. The adverse event profiles of the two groups were similar.

These findings demonstrate the potential utility of a therapy that restores CFTR function. Importantly, all patients in both placebo and ivacaftor groups were maintained on their existing treatment regimens (except hypertonic saline because it is not FDA approved for CF therapy). The improvements in lung function and the other clinically relevant endpoints in patients already receiving the existing CF care is a life-altering result. A similarly designed trial, also with encouraging results, was published for children ages 6-11 with CF and at least one copy of the G551D mutation. As encouraging as these studies are, patients with G551D CFTR mutations represent approximately 4% of current individuals with CF. The high cost of the drug may also be a barrier for some patients.

Reference

IVACAFTOR IN INDIVIDUALS WITH CF AND THE F508DEL CFTR MUTATION


(For non-subscribers to this journal, an additional fee may apply to obtain full-text articles.)

This article summarizes a randomized trial comparing ivacaftor to placebo in patients with CF who are homozygous for the most common mutation, F508del. The rationale for this study was that in vitro experiments showed increased CFTR activity in cells carrying CFTR with an F508del mutation that are treated with ivacaftor. The magnitude of response was not as great as in cells that carried CFTR with the G551D mutation. It was not known whether these smaller improvements in CFTR function would translate to clinically meaningful improvements for CF patients with F508del mutations.

The F508del mutation and the G551D mutation disrupt CFTR in different ways. F508del prevents correct folding of the newly synthesized protein; as a result, very little CFTR protein can reach the cell surface, although the small amount that does is functional. G551D-CFTR, however, folds normally and can be found at the cell surface but is cannot be "turned on" to act as a chloride channel. The drug ivacaftor aids in "turning on" the mutant G551D-CFTR. Because a small fraction of F508del reaches the cell surface, it was considered worthwhile to see whether a drug that enhances chloride conductance, such as ivacaftor, would be beneficial.

The results of the trial demonstrated in that ivacaftor had no substantial benefit to individuals with CF due to two copies of the F508del CFTR mutation. Of the 140 patients, 112 were randomized to receive ivacaftor at the same dose as the study by Ramsey et al (150 mg twice daily), and all were offered the drug as part of the open label extension. A 2.9 mmol/L lowering of sweat chloride was seen in the patients treated with ivacaftor compared to patients receiving placebo at 16 weeks (95% CI 5.6 - 0.2, P = 0.04). However, this evidence of improved CFTR function was not associated with any statistically significant difference in lung function, quality of life (once again assessed by CF specific questionnaire), pulmonary exacerbations, or weight gain.
If larger improvements in CFTR function were associated with clinically relevant and statistically significant improvements in outcomes such as lung function, why do small improvements in CFTR function not show at least some translation to clinical outcomes? Two explanations are possible. First, the study was not designed or powered to detect small differences in lung function. It is likely that the small magnitude of improvement in CFTR function, as evidenced by the lowered sweat chloride, would have to be present for a much longer time before any difference in lung function were apparent. Second, it is also possible there is a threshold to which CFTR function must be restored before a difference in organ function is realized. These considerations will be important as future therapies are investigated.

Importantly, this trial provides further evidence that ivacaftor has an acceptable safety profile. However, no drug, especially not a new drug, has zero risk of adverse events. The most commonly seen side effects were symptoms that could be considered part of the spectrum of CF disease, such as cough, sinus congestion, and pulmonary exacerbation. All of these events occurred at similar rates with the placebo group. There were slightly higher occurrences of rash and contact dermatitis in the ivacaftor group, and these symptoms should be monitored in patients receiving ivacaftor.

**CFTR GENOTYPE AND PROGNOSIS**


*(For non-subscribers to this journal, an additional fee may apply to obtain full-text articles.)*

This article from 2006 by McKone and colleagues addresses how genotype can be used to predict life expectancy in individuals with CF. As discussed in this issue's Commentary, these types of prediction are of limited utility to any single patient. There is great variability in lung function, in infections, in response to treatment, and in the myriad of other factors that influence prognosis. This variability is present even among individuals with identical genotypes. The consensus in CF care has been: "We can use genotype to predict whether a patient will be pancreatic-sufficient, or be pancreatic-insufficient and require enzyme replacement, but we cannot reliably predict lung function." This slightly older work, which analyzes outcomes from the US CF Patient Registry, remains the best effort to date to quantify the effect of genotype on lung function.

To carry out this analysis, the researchers divided all patients in the US CF registry between 1993 and 2002 into "low-risk" vs "high-risk" genotypes. Remembering that the genotype takes into account the mutation at both of the patient's *CFTR* genes, patients were defined as "low-risk" if either of their *CFTR* mutations fell into the functional classes IV or V (mutations that are associated with some residual CFTR function). Typically, patients with at least one class IV or class V mutation are pancreatic sufficient. Ninety-three percent of patients (n = 14,525) were characterized as "high-risk" genotype; the remaining 7% (n = 1,126) patients were characterized as a "low-risk" genotype. This retrospective study allowed the researchers not only to compare lung function, pancreatic status, and microbiology data, but also to look at survival. Those with the "high-risk" genotype, despite being on average younger, were 2.25 times more likely to die (95% CI 1.77-2.84) during the follow-up period than those with the "low risk" genotype. Of the 1,672 patients who died over the course of the study, the mean age of death for "high-risk" genotype was 24.2 vs. 36.7 for the "low-risk" genotypes (P < 0.001). It is difficult to tell what accounted for this increased risk of death, as the rates of pancreatic insufficient and the percentage of patients chronically colonized with *Pseudomonas aeruginosa* were higher and the body mass index was lower in the patients with the "high-risk" genotype. At first glance there is no difference in lung function between the two groups, although this likely is an artifact of the survival bias and older age of the "low-risk" genotype group. Even controlling for BMI, pancreatic function, *Pseudomonas aeruginosa* colonization, and lung function, *CFTR* genotype risk group was an independent predictor of survival.
If this study were done today, while the average ages of death would probably be higher for both groups, the difference between groups would remain. It is apparent that any residual CFTR function is associated with better survival. This is encouraging as we investigate CFTR-specific therapies such as ivacaftor. If the difference between a "low-risk" genotype and a "high-risk" genotype is associated with increased survival, then therapies that rescue CFTR effectively enough, can be given early enough, and can be sustained long enough, should convey survival benefit.

A problem with this study is that nearly 11,000 patients had a genotype that could not be classified as "low-risk" or "high-risk." That challenge is addressed in the CFTR2 article reviewed elsewhere in this issue.

DEFINING CFTR-RELATED DISORDERS


(For non-subscribers to this journal, an additional fee may apply to obtain full-text articles.)

As our understanding of CF and of the genetic changes that cause it have advanced, we are increasingly recognizing patients who have some features of CF but do not follow the more predictable disease course seen in patients whose CF was diagnosed at birth or shortly after. The term "atypical" CF has been used to describe CF diagnosed after childhood with milder organ system manifestations. This term is potentially misleading, as these patients often die from CF lung disease, although at a later age than those with more "typical" CF. The genotype of these patients would often fall into the "low-risk" category described in the McKone review. If one thinks of CF as a spectrum, with pancreatic-insufficient CF on one end of the spectrum and healthy people without CFTR mutations on the other end, some individuals will fall between these extremes. In addition to the atypical patients described above, some patients have symptoms that can be seen in CF and are a result of CFTR dysfunction but do not meet the diagnostic criteria for cystic fibrosis.

Bombieri and a group of mostly European CF experts addressed this group of patients in the article presented here. They define CFTR-related disorders (CFTR-RD) and present diagnostic algorithms to differentiate these individuals from those with CF. The clinical syndromes that fall into the CFTR-RD diagnosis include obstructive azoospermia resulting in male infertility (often labeled as congenital bilateral absence of the vas deferens or CBAVD), acute recurrent or chronic pancreatitis, or disseminated bronchiectasis. To fulfill these experts' diagnosis of CFTR-RD, a person would have to have one of these clinical syndromes without another organ system involvement, but with some evidence of CFTR dysfunction. The evidence for CFTR dysfunction can be provided two ways: either an abnormal physiologic test of CFTR function (nasal potential difference or intestinal current measurement) or genetic testing that identifies CFTR variants. However, by the current CF diagnostic criteria, a person with an organ dysfunction that had two CFTR mutations identified on genetic testing could be diagnosed with CF. The key difference is that not all genetic changes in CFTR are clearly known to be CF-causing mutations.

The authors note appropriately that most of the time the diagnosis of CF is straightforward. A diagnosis of CFTR-RD describes a group of disparate single organ system conditions that all have CFTR dysfunction. It should be noted that the diagnosis of CFTR-RD is separate from the condition cystic fibrosis related metabolic syndrome, as described by Borowitz et al in 2009. Cystic fibrosis related metabolic syndrome (CMRS) is a diagnosis given to newborns who screen positive for CF, who are asymptomatic, and do not meet diagnostic criteria for CF. Both conditions share the common scenario in which patients may be identified with CFTR variants of uncertain significance.

Reference

The Clinical and Functional Translation of CFTR (CFTR2) is an international consortium funded by the US CF Foundation with the goal of increasing the number of CFTR mutations that have well-classified disease liability. The project began by collecting patient data from national registries and from CF clinics in countries that did not have a national registry. In total, nearly 40,000 individuals with CF were collected from 27 countries, mostly in North America and Europe. From this patient population we compiled a list of the most frequently seen mutations in individuals with CF. Some 159 mutations were seen in nine or more alleles in the database, accounting for an allele frequency of greater than 0.01% (frequency of that specific mutation among CF chromosomes).

It is not enough to say that if a mutation is seen in a patient with CF, that it is a CF-causing mutation. The numerous examples in CF and in other genetic diseases of mutations misidentified as disease-causing are particularly concerning, as frequently CF mutation analysis is used as part of carrier screening and to help couples make reproductive decisions. To verify that these mutations are CF-causing, we analyzed the clinical consequences in patients from our database who carried the mutation. We used sweat chloride concentration as a marker of CF severity, comparing only patients who had the mutation on one chromosome with a mutation known to completely disrupt CFTR function on the other chromosome. We also performed functional analysis, based on the mechanism of each mutation. For the missense mutations and splicing mutations, this allowed a quantitative comparison (using wild-type CFTR as a reference). Finally, we compared the frequency of each mutation in the CFTR2 population with its frequency in the general population (from the 1000 genomes project), and its frequency in CFTR mutations carriers. Using these criteria, 127 of the 159 mutations met clinical (average sweat chloride greater than 60 mmol/L), functional (mutations that introduced a premature termination or resulted in less than 10% of wild-type CFTR RNA level, protein level, or protein function), and had no evidence of non-penetrance in CFTR mutation carriers or in the general population.

This definitively characterized list expands the number of CF-causing mutations from the 23 mutations agreed upon as part of the American College of Medical Genetics. Of the remaining mutations, 12 mutations could be declared not CF-causing, and the remaining 20 are indeterminate or of variable penetrance. A key finding reinforced by these mutations seen in individuals with CF but that failed to meet all of our criteria to be classified as CF-causing, is that mutations lie along a spectrum as well. Especially for the mutations associated with residual CFTR function, there is no clear line between a fully penetrant CF-causing mutation, or a mutation that causes "atypical CF" or one that is associated with CFTR-RD.

The CFTR2 project is ongoing and will continue to analyze mutations seen in individuals with CF. Almost 2000 CFTR mutations have been described, so considerable work remains to be done. This data is publicly available via the CFTR2 website, which will be updated as more patient data are collected and further functional testing is completed. As discussed in the other articles in this issue, correct characterization of CFTR mutations has important implications to therapy selection, to prognosis, and to CF diagnosis.

Reference
1. www.cftr2.org

© 2014 JHUSOM, IJHN and eCysticFibrosis Review

Presented by JHUSOM and IJHN in collaboration with DKBmed.