Modifers of Cystic Fibrosis Disease

In this Issue...

Although the disease-causing gene for cystic fibrosis (CF) was identified in 1989, subsequent studies have confirmed that the severity of many disease manifestations in CF, particularly lung disease, is largely independent of the CFTR genotype. Specifically, patients with identical mutations in CFTR have been shown to have dramatically different disease courses. Identifying non-CFTR modifiers of CF disease is crucial to ameliorating disease burden. Recent studies have identified both genetic and environmental modifiers of CF disease, leading to opportunities to counsel patients on exposures and to enhance their understanding of CF pathophysiology.

In this issue, we discuss recently identified modifiers of CF disease, and the ramifications for future clinical management and therapies.

LEARNING OBJECTIVES

After participating in this activity, participants will demonstrate the ability to:
- Describe the importance of modifiers other than the CFTR genotype in disease manifestations in patients with cystic fibrosis (CF),
- Discuss the various approaches used to identify modifiers of CF disease,
- Recognize the need for additional research to translate identified genetic modifiers into an improved understanding of CF pathophysiology.

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Guest Author's Disclosures
The use of genetic information in clinical medicine is imminent, with the cost of DNA sequencing is decreasing exponentially. Clinicians will be increasingly asked to provide their interpretation of genetic data, regardless of the medical or scientific merit of conducting such testing. Interpretation of these data will rely on findings from previous studies of candidate genes, linkage, and genome-wide association. Thus, it is important for clinicians to recognize the benefits and limitations of genetic studies, as well as the importance of environmental studies, in the genomic era. Despite cystic fibrosis (CF) being a monogenic disorder, ample evidence has shown that modifiers other than the CFTR genotype substantially alter outcomes for many disease traits.
Although the financial cost of genetic studies is on the decline, genome-wide association studies frequently involve thousands of subjects, cost millions of dollars, and can take many years to complete. Prior to embarking on such research, estimating the genetic contribution to a variation in a disease trait (i.e., heritability) is essential. These heritability estimates are frequently used prior to conducting genetic analyses, to select disease traits with strong genetic components. By the same token, it is useful to know the environmental contribution to the same trait. In the first article reviewed in this issue, Collaco and colleagues demonstrate that half of the variation seen in CF lung function can be attributed to genetic factors, with the remainder resulting from environmental factors. That finding provides the justification for studying both genetic and environmental modifiers of CF lung disease.

Although the processing of biological samples and analyses of genetic data can be time-consuming, obtaining high-quality data for an entire genome may be easier than obtaining an accurate, comprehensive assessment of an individual’s environmental exposures. This remains problematic, as currently, it may be easier to alter the patient’s environment than to alter their genes to improve outcomes. As in many diseases, environmental studies in CF are limited to examining one or a handful of candidate environmental exposures. Because of confounding results, it is difficult to definitively state that a specific environmental exposure causes a particular outcome. In the second article discussed in this issue, Schechter reviews the breadth of environmental studies conducted in patients with CF. A common thread is the association of lower socioeconomic status with worse CF outcomes—a correlation that has relevance in societies with increasing income inequality and decreasing public health insurance budgets. In addition, since genes do not operate in a vacuum, as genetic studies proceed, it is logical to examine whether replicated genetic modifiers operate through known environmental modifiers (i.e., gene-environment interactions). As patients present with “unfavorable” genetic polymorphisms, assessing environmental factors known to interact with the gene in question is essential to assessing the true risk for a genetic polymorphism.

One method of identifying genetic modifiers of disease is to select candidate genes, or genes in which variation could be plausibly linked to outcomes. In the third article reviewed, Bartlett and associates determine whether polymorphisms in selected candidate genes are associated with CF-related liver disease. Their approach reflects a key aspect of many recent genetic studies—that is, replication. Given the large numbers of genes that are frequently examined in genetic studies, the probability of detecting a false association increases with the number of statistical tests performed. Replication of associations helps to reduce the risk for reporting a false association. With replication, Bartlett and colleagues identify one polymorphism in the α1-antitrypsin gene that is associated with CF-related liver disease, as well as non–CF-related liver diseases, including biliary atresia, viral hepatitis, and alcoholic liver disease. Thus, since genetic modifiers of disease may play a role in multiple diseases, including CF, strong gene associations in other diseases with manifestations similar to CF phenotypes may be promising candidates for study in patients with CF.

Another means for replicating candidate gene associations is meta-analysis. This statistical approach involves combining the results of separate, previously published studies to gain greater power to detect effects. In the case of the mannose-binding lectin 2 (MBL2) gene, multiple studies have been conducted to determine its association with patient outcomes in CF, with differing results. As most of these studies have enrolled relatively low numbers of subjects, they may have been underpowered to detect effects. In the fourth article reviewed, Chalmers and coworkers showed associations between “insufficient” MBL2 genotypes and earlier acquisition of Pseudomonas aeruginosa, reduced pulmonary function, and increased risk for mortality. The investigators included a total of 16 studies; however, it should be noted that meta-analyses rely on published data, and because there is a bias against publishing negative results, there may be an overrepresentation of studies with associations in Chalmers’ meta-analysis.

A burgeoning area of research is genome-wide association analysis, an agnostic approach to identifying gene modifiers, as it does not assume any prior knowledge about a specific gene’s function. The fifth article reviewed in this newsletter provides the first published genome-wide association study in CF. Wright and colleagues identified 2 loci of interest in chromosomes 11 and 20 using genome-wide association and linkage techniques in 3 separate samples of CF subjects from North America. Differences in genotype at the chromosome 11 locus were estimated to be associated with a 5.1 ± 1.9% difference in forced expiratory volume in 1 second (FEV1) percent predicted, whereas the chromosome 20 locus contributes between 4% and 46% of the variation in lung function.
among siblings with CF. Although the identified loci are genetic modifiers of CF lung function, as with any genome-wide analysis, additional work is required to map the variation in lung function to a specific gene.

In summary, the field of modifier studies in CF is evolving and progressing rapidly. Because of the large numbers of CF patients, families, and clinicians willing to participate in research, as well as strong foundation backing, CF is one of the paradigms for studying modifiers in a monogenic disorder. It is likely that the lessons learned in modifier research in CF may be applicable to studies of other monogenic disorders.

Collaco and colleagues attempted to determine the relative contributions of environmental and genetic factors to cystic fibrosis (CF) lung function using a study sample derived from the CF Twin and Sibling Study. The investigators generated estimates of relative contributions by examining differences in lung function among 62 pairs of family members living in the same household or living in different households. They replicated their estimates of environmental contributions in a separate population of 40 pairs of siblings.

The authors of the study examined monozygous twins (100% gene sharing) and dizygous twins/siblings (~50% gene sharing) assumed to have similar common environmental exposures from living in the same household, then examined the same individuals after they moved to different households. Even monozygous twins living together were found to have differences in forced expiratory volume in 1 second (FEV$_1$), highlighting the contribution of unique or stochastic (random) environmental factors to an individual's lung disease (eg, 1 twin contracts influenza). These differences in lung function increased after the twins moved into different households, emphasizing the fact that sharing a common environment also plays a role in lung disease. Lastly, the differences in lung function among family members were amplified in dizygous twins/siblings compared with monozygous twins, highlighting the genetic contribution to CF disease. The figure below summarizes the key findings of this study, namely that genes and the environment contribute equally to CF lung function, and most of the environmental contribution is mediated through unique or stochastic environmental factors, rather than through common environmental factors. Collaco and associates provide quantitative estimates of the contribution of various factors to lung function as well, specifically genetic (50%), common environmental (14%), and unique environmental/stochastic (36%).
With the increasing feasibility of conducting large-scale genomic studies to identify potential gene modifiers of CF disease, the need to determine the genetic contribution to disease severity is crucial, even in a monogenic disorder. Traditionally, heritability estimates or other family-based approaches have informed funding agencies and researchers prior to proceeding with costly, time-consuming genetic studies. For example, a trait with a low estimate of heritability or genetic contribution may not be a good candidate for genetic studies. The researchers provide justification for pursuing studies of both genetic and environmental modifiers of CF lung disease, as the relative contributions of genetic and environmental modifiers were found to be equal. In addition, determining the contribution of the shared vs the common environment may be helpful in assessing the risks for patients with CF who share environments, such as a household, a CF center clinic, or a summer camp. The relative importance of unique factors found by the authors may help to inform future studies of gene-environment interactions in CF lung disease.

Abbreviations: CF, cystic fibrosis; DZ, dizygous; FEV₁, forced expiratory volume in 1 second; MZ, monozygous; Sib, sibling.

Schechter provides a concise, yet comprehensive, review of the CF literature on environmental modifiers, including socioeconomic status (SES), gender, race/ethnicity, secondhand smoke exposure, air pollution, infectious agents, mental health, nutritional status, adherence, and differences in care provision.


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A variety of domains of lower SES have been associated with worse outcomes in patients with CF, including manual parental occupations, Medicaid vs private insurance, and median family income. Both female gender and non-white race/ethnicity have been associated with worse patient outcomes, but it has been difficult to determine the relative contributions of genetic influences vs sociocultural influences to worse outcomes. Secondhand smoke exposure has been associated with lower lung function and may be an important confounder of the relationship between lower SES and patient outcomes, as secondhand smoke exposure is also associated with lower SES. Air pollution independent of SES has been demonstrated to be associated with increased pulmonary exacerbations and decreased FEV$_1$ within the US CF population. In terms of infectious agents, acquisition of *Pseudomonas aeruginosa* or *Burkholderia cepacia* complex has been associated with more rapid decline in lung function. Of note, more recently published data not included in Schechter's review highlight increased morbidity and mortality associated with the acquisition of methicillin-resistant *Staphylococcus aureus*. Anxiety and depression have been associated with lower lung function and worse nutritional status. Nutritional status is highly correlated with lung function in patients with CF, although it remains unclear whether the predominant effect is poor nutrition leading to worse lung function or vice versa. Both patient nonadherence and variation in provider practices are likely to lead to worse outcomes; research is ongoing to determine the relative magnitude of these exposures compared with other environmental modifiers.

As the cost of performing genomic sequencing decreases exponentially, comprehensive genetic information will be easier to collect than even limited environmental datasets for modifier studies. However, as Schechter points out, "[A]n understanding of nongenetic factors provides more immediate tools for improving disease outcomes." Many of the environmental factors leading to poor outcomes do not require the development of new therapies but, as in the case of nonadherence and variation in provider care, merely improved application of existing therapies. It should be noted that the environmental studies have reported on associations with poor outcomes, not necessarily on causal factors. As is true for genetic modifiers, replication of association for environmental factors in independent populations is needed to confirm the presence of a specific modifier. Schechter also highlights the confounding nature of SES within many of the studies on which he reports, emphasizing the need for studies that provide robust results after including a reasonable panel of confounders, including gender, race/ethnicity, and SES. Of fundamental importance is the fact that many of the identified environmental modifiers also affect many other non-CF chronic diseases. Improving outcomes in patients with CF may mean borrowing successful strategies used in the management of other chronic diseases.

Commentary References


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**GENETIC MODIFIERS OF CYSTIC FIBROSIS LIVER DISEASE**


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Bartlett and collaborators attempted to assess whether 9 polymorphisms in 5 selected candidate genes were associated with the development of severe CF-related liver disease (CFLD) with portal hypertension. The genes (α1-antitrypsin or α1-antiprotease [SERPINA1], angiotensin-converting enzyme [ACE], glutathione S-transferase [GSTP1], mannose-binding lectin 2 [MBL2], and transforming growth factor-β1 [TGFB1]) were selected on the basis of prior study in CFLD. A case-control design was used because of the low frequency of severe CFLD among patients with CF (3% to 5%). The first phase of the study assessed the
frequency of specific polymorphisms in individuals with CFLD (cases) vs controls diagnosed with CF but not with CFLD. The second phase of the study attempted to replicate associations observed in the first phase using separate case and control samples.

In the first phase of their study, the investigators examined the frequency of polymorphisms in 124 cases of CFLD vs 843 CF control subjects from across North America. This first phase demonstrated associations with CFLD and the SERPINA1 Z allele and the TGFB1 codon 10 CC genotype. The second, or replication, phase, which was conducted among an independent sample of 136 cases and 1088 controls, confirmed the SERPINA1 Z allele association but not the TGFB1 codon 10 allele. A combined analysis of both phases of the study demonstrated a 5-fold increased risk (odds ratio [OR], 5.04; 95% confidence interval [CI], 2.88, 8.83; \( P = 1.5 \times 10^{-8} \)) of developing CFLD with portal hypertension associated with the SERPINA1 Z allele.

This study highlights an example of a very successful genetic modifier study. By carefully selecting candidate genes for study, Bartlett and colleagues have identified an allele with a substantial association (OR, 5.04), which is large for a genetic modifier in a monogenic disorder. By identifying a polymorphism known to be associated with the development of other liver diseases, this study serves as an additional reminder that a single genetic modifier may increase the risk for multiple diseases. In the future, clinicians caring for patients with CF may be able to leverage this knowledge by utilizing genetic tests already in use for these other diseases, thus reducing development and test costs. It is also not unreasonable to consider future trials of therapies for patients with CF based on successful management strategies used in other liver diseases.

CANDIDATE GENE APPROACHES FOR DETECTING MODIFIERS IN CYSTIC FIBROSIS


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Mannose-binding lectin (MBL) is an important component of innate immunity in the lungs. After forming a multimer, MBL binds to sugar moieties on bacteria and activates the complement pathway. Variants in *MBL*—the gene that encodes MBL—can disrupt the formation of multimers, leading to reduced levels of the functional form of MBL and an excess of monomeric forms of MBL. MBL insufficiency has been associated with a number of infectious conditions. As CF lung disease is characterized by a predisposition to chronic bacterial infection, numerous studies have evaluated whether variation in MBL is associated with differences in severity of lung disease among patients with CF. Although some studies (1-7) have reported a correlation between MBL insufficiency and lower lung function measures, other studies have not (8-12). To address the apparent lack of consensus among the published studies, Chalmers and colleagues conducted a meta-analysis of published studies regarding the influence of MBL variation on CF lung disease severity. The authors selected 16 original publications that evaluated the association between variation in the level of MBL and an accepted measure of lung disease severity. In their review, the researchers required that MBL levels be assessed by using serum measurement and/or by genotyping alleles known to cause variation in MBL levels. They also required that CF in the study population had been confirmed by the *CFTR* genotype and that *MBL2* genotype distributions were tested for equilibrium using the Hardy-Weinberg equation.

Chalmers and coworkers evaluated 6 traits that have been correlated with MBL insufficiency: (1) *P. aeruginosa* infection status, (2) age at infection with *P. aeruginosa*, (3) *B. cepacia* infection status, (4) FEV1, (5) forced vital capacity (FVC), and (6) survival. As the studies varied in the outcomes evaluated, different numbers of studies contributed to the meta-analysis for each trait. In 4 of the studies, *MBL2* genotypes associated with MBL insufficiency correlated with earlier acquisition of *P. aeruginosa* (mean difference, 2.83 years; 95% CI, 1.63 to 4.03 years; \( P < .0001 \)). Reduced pulmonary function measures including both FEV1 (mean difference, -13.83; 95% CI, -6.69 to -20.97; \( P = 0.0001 \)) and FVC (mean difference, -13.31; 95% CI, -2.97 to -23.66; \( P = .01 \)) also correlated with MBL deficiency. The latter effect was
confined to adult patients (>18 years of age), however, as 5 studies involving pediatric patients failed to demonstrate a significant association. Finally, the association of insufficient MBL2 genotypes with increased risk for mortality or need for lung transplantation was noted (OR, 2.36; 95% CI, 1.06 to 5.22; P = .03.)

The meta-analysis conducted by Chalmers and colleagues confirms the role of MBL insufficiency as a contributor to the severity of CF lung disease. Although meta-analytic studies are dependent on the number and quality of published reports, it appears that sufficient consensus is present to conclude that variation in MBL modifies infection, lung function, and longevity in persons with CF. The major limitation of these conclusions is the general bias to report positive correlations rather than negative findings. Although these findings demonstrate the value of the candidate approach to detecting genetic modifiers of CF, Chalmers and collaborators illustrate the importance of reconciling findings when numerous partially conflicting studies have been published. Therapy for MBL insufficiency may have limited efficacy in the CF population, as it affects only 10% to 15% of individuals with the disease. MBL replacement may be a viable option for selected CF patients who manifest early infection with P. aeruginosa infection, however, in order to increase lung function and improve survival.

Commentary References


GENOMIC APPROACHES FOR DETECTING LUNG DISEASE SEVERITY IN CYSTIC FIBROSIS


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is limited to our current understanding of CF pathophysiology. Searching for modifier genes throughout the genome without a priori hypotheses regarding function is possible using the techniques of association and linkage. Association methods enable the detection of common genetic variants that contribute to disease variation. Unrelated individuals stratified by a common trait (eg, FEV$_1$) are tested for differences in the frequencies of a large series of genetic variants called single nucleotide polymorphisms (SNPs) that are distributed across the entire human genome. Correlation is sought between the genetic variants (alleles) in 1 SNP (or a cluster of physically close SNPs) and clinical values. For example, an SNP may have 2 alleles: (1) a more common, or major A allele and (2) a less common, or minor G allele; an association would be sought if the A allele correlated significantly with lower FEV$_1$. By convention, the G allele will be correlated with higher FEV$_1$. Association by design is usually not able to detect genetic variation in which the frequency of the minor allele is <5% in the population. In addition, most of the common variants detected by association have a relatively small effect on trait severity. Family-based studies that use siblings are designed to detect rare variations that can have small or large effects on trait severity. This is accomplished by searching for regions of the genome that are shared in siblings with a similar severity of disease (and vice versa). Such regions are said to be linked to the trait under study. Wright and colleagues used both association and linkage approaches in 2494 unrelated CF patients and 486 CF sibling pairs to search for modifiers of FEV$_1$ in CF. The patients were derived from 3 separate studies: the Genetic Modifier Study (GMS), the Canadian Genetics Study (CGS) of CF, and the CF Twin and Sibling Study (TSS). More than 600,000 SNPs were typed in these patients using the Illumina 610K assay.

Genome-wide analysis revealed 7 loci demonstrating association at the suggestive level of significance in the combined GMS and CGS samples of 2494 unrelated CF patients. When the analysis was restricted to 1978 patients with identical CFTR genotypes ($F508del$ homozygotes), 1 locus on chromosome 11 involving an SNP between the $EHF$ and $APIP$ genes exceeded the threshold for significance at the genome-wide level ($P = 3.34 \times 10^{-8}$). The same SNP demonstrated association with lung function ($P = .006$) in CF siblings from the TSS, thereby replicating the finding in the GMS and CGS samples. In addition, linkage exceeding the threshold for genome-wide significance was observed on chromosome 20 ($\log_{10}$ odds = 5.03). Association analysis of the critical region of linkage using the GMS and CGS patients identified an SNP located in the middle of the linkage region, approximately 200,000 base-pairs from the nearest $CBLN4$ gene. Four other genes are located in the critical region of linkage.

Wright and colleagues report the successful application of genome-wide methods to detect loci associated with variation in lung function in CF. Bioinformatic analysis indicates that the $EHF$-$APIP$ region likely harbors a common DNA variant that affects the expression of 1 or both genes. $EHF$ encodes a transcriptional factor expressed in the lung, and it had previously been suggested as a potential modifier of CF lung disease. $APIP$ encodes an APAF-1-interacting protein that plays a role in apoptosis. Both genes are expressed in the respiratory tract. Expression analysis suggests that increased expression of $APIP$ appears to be associated with lower lung function, suggesting that inhibition of apoptosis may worsen lung disease in persons with CF. The 5 genes under the chromosome 20 linkage region are expressed in the respiratory tract, with 2 of these genes having been implicated in lung defense ($MC3R$ and $CASS4$). The discovery of these modifier loci focuses attention on pathologic mechanisms, such as apoptosis and neutrophil accumulation, that in the past have received little attention.

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