Who Modifies the Modifiers: A High-Resolution View of the Genetic Modifiers of Cystic Fibrosis

Cystic fibrosis (CF) is an autosomal recessive disease caused primarily by mutations of the gene encoding the CF transmembrane receptor (CFTR) (1, 2). A defective CFTR protein leads to systemic dysfunction involving the sinopulmonary, gastrointestinal, pancreatic, and reproductive systems, with the primary cause of morbidity and mortality being respiratory failure. Although CF is often regarded as a single-gene disorder, the severity of lung disease varies widely, even among those with the same CFTR variant. A significant proportion of variability is explained by modifier genes (3). Even in an era of novel CFTR modulators, about 10% of CFTR mutations are not compatible with modulator therapy (4), and response to therapy may be variable (5); therefore, the identification of modifier genes and pathways could lead to potential targets for further therapies.

Although CF fits into the category of a Mendelian disease, with rare alleles causing large effects, whether the genetic variants that modify CF are common or rare and how these variants contribute to the spectrum of disease is not clear. To date, most studies use genome-wide association studies (GWASs) based on genotyping arrays that assay common variants to explain the variability of complex traits. When GWASs fail to identify an association, either the effect size of a common variant is too small to be detected at a genome-wide–significant level or a rare variant with a strong effect size is too rare to be identified by using a GWAS array (6). Using a larger sample size, multiethnic samples, and whole-genome sequencing to identify rare variants can improve the likelihood of identifying new associations.

In this issue of the *Journal* (pp. 1324–1333), Zhou and colleagues present the largest integrative genomic analysis to date in patients with CF, including 7,840 individuals (7). By generating new whole-genome sequencing samples from three U.S. studies and using a statistical technique called imputation that uses sequencing data to "fill in" gaps from GWAS array data from prior published GWAS studies, the authors were able to present the highest-resolution view to date of how non-CFTR modifier genetic variants can impact lung disease in people with CF.

The primary response variable used to quantify the severity of lung disease in this study was the Kulich normal residual mortality-adjusted lung disease phenotype (8). This validated quantitative phenotype is derived from CF-specific FEV_1 percentiles (9), allowing for comparison of lung function relative to other patients with CF of the same age, sex, and height and accounting for mortality attrition due to severe disease.

The overall analysis, which included participants from prior GWASs, identified six genome-wide–significant loci (labeled by genes closest to the loci *MUC4/MUC20*, *SLC9A3/CEP72*, HLA class II, *APIP/EHF*, *CHP2/PRKCB*, and *AGTR2/SCL6A14*) (10, 11). Although all these loci were identified in previous GWASs, a strength of this paper is how the authors performed a more detailed granular analysis at these loci. The authors conditioned on top-ranked SNPs, revealing regional signals for significant secondary SNPs at two loci (chr5p15.33: *SLC9A3/CEP72* and chr11p13: *APIP/EHF*), which independently associate with lung disease severity. Although the effector gene at these loci is not known, the closest genes suggest some potential mechanisms. *SLC9A3* encodes an Na/H exchanger and is involved in regulating airway surface liquid pH; *EHF* is a transcription factor involved in epithelial differentiation (12); and *APIP* is involved in apoptosis and methionine salvage (13). The discovery that there are multiple significant SNPs at these two loci highlights the complexity of genetic associations and will be informative to evaluate in future studies.

The authors also use pathway analysis to implicate genetic variants in genes related to lung development and branching morphogenesis as modifiers of CF lung disease. This work aligns with current thinking of the importance of early life in chronic obstructive pulmonary disease, which has also been supported by GWASs (14). The authors specifically cite dysanapsis, a mismatch of airway caliber to lung size, as a possible mechanism of CF lung disease related to lung developmental pathways. Dysanapsis was first proposed to explain spirometric variability in healthy adults (15); it has recently been quantified using computed tomography and associated with chronic obstructive pulmonary disease risk (16) and childhood asthma. However, not all abnormal lung development represents dysanapsis. In addition, reactivation of developmental pathways may be necessary for lung repair, suggesting some variability in lung disease severity may instead be evidence of abnormal repair to repeated injury in CF and potentially other lung diseases characterized by repeated injury.

In addition to notable strengths, this study is not without limitations. Most notably, there were no new significant loci reported. Even though this is the largest CF genetic association study to date, evidence from genetic studies of other complex traits indicates that there are likely other genetic variants to be discovered responsible for modifying CF lung disease that have lower allele frequency or smaller effect sizes but still could identify new targets for therapy. The authors do note that effect sizes in the individual cohorts were ordered, suggesting that age for example may need to be considered in future studies. Other types of variants, such as copy number variation and variable number of tandem repeats, which may also modify the effects of CFTR on lung function (17), are not fully considered in this study. This study was also completed before the widespread use of CFTR modulator therapy, and the effect of a rescued CFTR protein on lung

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function and identification of modifier genes is yet to be evaluated. Finally, the genetic association does not identify the causal variant, effector gene, cell type, and biologic function in context.

In summary, this study provides a detailed, genome-wide search for variants that affect lung disease in CF. How these variants affect specific genes to modify the effect of CFTR and whether perturbation of these genes or related pathways could restore lung function in the age of novel CFTR modulators is a rich area for future investigation. ■

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Moving Beyond $PM_{2.5}$ Mass to More Effectively Protect Health

Particulate-matter air pollution is widely recognized as a major public health threat, causing millions of excess deaths per year globally (1). To better protect public health, the U.S. Environmental Protection Agency (EPA) is presently considering lowering the long-term limit on permissible levels of atmospheric fine particulate matter mass (particles <2.5 μm in aerodynamic diameter [$PM_{2.5}$]) from an annual average of 12 μg/m^3 to $\leq$10 μg/m^3. The American Thoracic Society has recommended setting the long-term annual average standard at 8 μg/m^3 and lowering the 24-hour average limit to 25 μg/m^3 (2). As levels decrease in the United States and around the world, meeting more stringent $PM_{2.5}$ mass standards will likely become more challenging, so there is an imperative to prioritize the most health-impactful particle components. The EPA and other health agencies should therefore also be investigating the potential setting of more specific particulate matter standards to focus more efficiently on regulating the most toxic particles within the $PM_{2.5}$ mass.

In the 1970s, governments around the world regulated particulate matter in bulk by putting a preweighed filter in front of what was basically a vacuum cleaner motor and measuring the total suspended particulate matter (TSP) added to the filter over a 24-hour period, regardless of particle size. For all the crudeness of the measurement method, a correlation was found between TSP and mortality by Lave and Seskin (3) and others.

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