to understand how these inhalants may interact to cause new or more severe effects on human health (Figure 1).

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Idiopathic Pulmonary Fibrosis Genetic Risk Factors, Function, and Mechanisms? The Concepts Are Starting to Gel

Patients with pulmonary fibrosis are more likely to carry certain alleles, including variants for the genes MUC5B (mucin 5B) (1) and DSP (desmoplakin) (2). However, in a world where 10% of the general population also carries these alleles, which at-risk carriers develop disease and why remains mysterious. In this issue of the Journal, Borie and colleagues (pp. 1259–1270) shed light on the case, asserting that genetic variation in MUC5B and DSP tips the scales toward fibrosis development via altered gene expression and DNA methylation (3). Their in silico approach to testing functional mechanisms highlights a novel method for future discovery.

Products of both genes assist the function and structure of the lung epithelium. First, MUC5B covers the respiratory epithelium in a protective jelly-like barrier. It provides the environment for optimal mucociliary clearance of pathogens and mediates activation of macrophages and other members of the innate immune response, including neutrophils and eosinophils (4–6). Patients with idiopathic pulmonary fibrosis (IPF) are more likely than those without IPF to possess a promoter SNP, rs35705950, which increases the constitutive
expression of MUC5B in the lungs (7, 8). The DSP gene codes desmoplakin, which adheres one airway epithelial cell to another via desmosomes, maintaining integrity of the epithelium under stress. In patients with IPF, the intron 5 variant rs2076295 for DSP is more frequently found and associated with decreased DSP expression in the lungs (2). These genetic polymorphisms affect the function and structure of the epithelium, supporting the hypothesis that epithelial cell injury and abnormal repair are necessary for the genesis of IPF.

Yet IPF risk alleles do not predict disease development. Risky gene expression can be modified through epigenetic mechanisms, including DNA methylation, nucleosome remodeling, histone modifications, and microRNA. Emerging knowledge of such epigenetic gene–environment interactions validates master clinicians who observe tobacco, air pollution, inorganic dust, and other environmental exposures more frequently in patients with IPF and who link these exposures to the evolution of disease (9). Indeed, differential DNA methylation in the MUC5B promoter region and promoter variant rs35705950 has been previously shown in patients with IPF (10). This targeted analysis set the stage for the present study.

In this issue of the Journal, Borie and colleagues (3) investigate the function of an expanded set of 10 common genetic IPF risk loci. They use high-quality contemporary multiomic data from the NHLBI-sponsored Lung Tissue Research Consortium. The analysis includes genetics, transcriptomics, and methylymethylation from the lung tissue of up to 202 control subjects and 345 individuals with IPF. Genome-wide DNA methylation and RNA sequencing were used to map expression quantitative trait loci (eQTL), those genomic loci that explain variance in expression of mRNA, and methylation quantitative trait loci (mQTL), those genomic loci that affect methylation patterns of cytosine–phosphate–guanine (CpG) DNA sequences. Having generated maps of these hotspots for gene expression and methylation variation, the investigators proceeded to use a novel probabilistic computational method, eQTL and genome-wide association study causal variant identification in associated regions (eCAVIAR) (11), to overlay the data and thus demonstrate colocalization. Colocalization via eCAVIAR of the genetic and eQTL signal for the MUC5B and DSP risk variants was outstanding. Genetic and mQTL signals also colocalized strongly for both MUC5B and DSP risk variants, suggesting that both genetic and epigenetic factors regulate gene expression. The alternative T allele for MUC5B was associated with higher methylation; similarly, higher methylation at a CpG site within DSP was associated with the presence of the alternative G allele for rs2076295. The other eight risk loci did not appear to have clear regulatory roles in this analysis.

One of the most novel findings comes from the luciferase assay testing in MUC5B, which was performed to further test the transcriptional activity of the MUC5B locus. This demonstrated that one CpG island resides in a repressor functional element. Finding the putative on or off switch strongly supports a function for MUC5B.

Although an important contribution, the study has its limits. The authors’ mQTL colocalization analyses are interpreted to mean that age, sex, and smoking do not significantly influence mQTL results. These subgroup analyses are limited by power. In addition, the ancestry of the cohorts must also be taken into account to understand the impact of rare variants in non-White populations. Whole-lung tissue, as discussed by the authors, also has its limits in examining the impact and function of rare cell populations obscured by common cell types.

Genetic variability may confer risk or deliver protection depending on disease context. Interestingly, although associated with higher attributable risk for developing IPF, the T allele MUC5B SNP predicts longer survival with IPF (12). Perhaps the high prevalence of these supposedly deleterious alleles is due to a competitive survival advantage, conferring some benefit after IPF has developed. Recently we learned that carriage of the T allele of MUC5B rs35705950 protects against the development of severe coronavirus disease (COVID-19) (13), and at the protein level, COVID-19 survivors with this same MUC5B T allele have higher plasma concentrations of MUC5B than nonsurvivors (14). Optimal mucin production may therefore be a trade-off, with high mucin production delivering protection from respiratory pathogens via enhanced host mucosal defense but conferring risk for IPF development in the long term.

The genetic complexity is highlighted when we consider the interacting contribution of desmoplakin and the DSP polymorphism. Reports on the rs2076295 DSP polymorphism straddle the chronic obstructive pulmonary disease (COPD) and IPF literature and are fascinating for their descriptions of opposing effects, with the high-expressing major T allele conferring risk for COPD (15) and progression of emphysema (16) and the low-expressing G allele associated with IPF (2). Here too, a trade-off may exist, with low concentrations of DSP adding risk for IPF in aged individuals but also protecting against the development of COPD and progressive emphysema.

Novel genetic and epigenetic therapeutics for IPF could rebalance this complex genetic instrument when it falls out of tune in old age. Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 (CRISPR-associated protein 9) genome editing has already been used in primary airway epithelial cell lines to modify the DSP risk variant (17). Epigenetic modification also holds promise. Understanding the causes and cures of IPF remains one of the great challenges of pulmonary medicine. But as the information gels, we move one step closer.

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Rare Genetic Variants Provide Protection for Obstructive Sleep Apnea

Obstructive sleep apnea (OSA) is an extremely common condition worldwide. OSA was shown many years ago to aggregate in families (1). Although this has been known for decades, progress in identifying relevant gene variants has been slow. This likely reflects both inadequate sample sizes and the etiological heterogeneity of OSA, with multiple risk factors that are each likely influenced by many genes.

Multiple approaches to identifying gene variants have been used, including candidate gene studies (which have been very underpowered [2]), family-based linkage studies (3) (which identified LOD scores below accepted ranges even for suggestive significance), and genome-wide association studies. Two different genotyping approaches to genome-wide association studies have been used: case-control analysis on the basis of clinical diagnosis (4) and quantitative trait analysis using measures from overnight sleep studies (e.g., the apnea–hypopnea index [AHI]) (5) shown to be heritable (6). The latter has been facilitated by conducting sleep studies in population-based cohorts. However, this approach can be challenging because there may be a only small subset of individuals with clinically meaningful OSA (5). There are also questions about whether subjects identified in the general population are representative of individuals who present clinically (7).

Although these efforts have begun to identify reproducible gene variants related to OSA, all variants together explain only a small fraction of the estimated heritability (4). Given that prior analyses have typically focused on common genetic variants (e.g., minor allele frequency [MAF] > 5%), one possible explanation for this “missing heritability” is associations with rare variants that have a lower prevalence (e.g., MAF < 5%). Although each individual variant is rare, there are a lot of them. In other complex traits, rare variants have been shown to explain a proportion of the missing heritability (8), supporting their potential role in OSA.

To assess the effect of rare variants, the study of Liang and colleagues (pp. 1271–1280) in this issue of the Journal (9) takes an interesting and informative multistage approach, leveraging major resources assembled by the highly innovative Trans-Omics for Precision Medicine (TOPMed) program sponsored by the NHLBI. Stage I involved linkage analysis for AHI in 487 European Americans from 118 families in the CFS (Cleveland Family Study) (1). The highest linkage peak was a suggestive association (LOD = 2.31) on chromosome 7q31. The investigators then used a number of filtering strategies and both gene-based burden tests and sequence kernel association tests to identify rare variants and implicated genes in the 20-cM region centered on the linkage peak. Although a number of different rare variants were identified, the most significant gene was CAV1 (Caveolin-1), which contained 21