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Omics and Lung Function: A Need for Integration

DNA methylation (DNAm) plays a role in a wide range of biological processes, including regulation of gene expression, reproduction, and development, and in chronic diseases and aging (1). The development of methodologies allowing the rapid and low-cost assessment of DNAm has enabled epigenome-wide association studies (EWASs) in large population studies that have increased our understanding of both the effect of environmental exposures on the methylome and the role of methylation in many diseases (2).

Maternal tobacco smoke exposure has shown highly specific changes in the offspring's epigenome at birth (3) that persist for decades (4). DNAm is a biomarker of tobacco smoke exposure (5) and is predictive of future asthma (6) and chronic obstructive pulmonary disease (COPD) (7). DNAm differences at birth have been shown to predict lung function growth trajectories (8) and to be associated with lung function and lung function decline in adulthood (9).

In this issue of the *Journal*, Lee and colleagues (pp. 321–336) describe the largest multiethnic EWAS of cross-sectional lung function to date in more than 17,500 individuals (Figure 1) (10). The differential methylation of 1,297 CpGs was associated with FEV_1, FVC, or FEV_1/FVC (after adjusting for technical experimental factors, estimated cellular composition, genetic ancestry, and smoking). Of these, 1,240 were newly described and 73 related to COPD. When comparing across ancestries, 294 lung function associated CpGs were unique to European or African ancestry, and 395 CpGs were unique to never- or ever-smokers. A key finding was that associated methylation marks were enriched for transcription factors, point toward accessible chromatin, and a druggable epigenome.

A major strength of this multiethnic study is the interrogation for functional and biologic relevance through gene expression, causal modeling, and colocalization efforts. Although limited by lack of longitudinal lung function modeling and limited assessment of lung tissue, this careful and comprehensive analysis provides a template for further investigations.

Given that DNAm is influenced by cell type, genetic variation, and environmental exposures, the large number of CpG sites associated with lung function is to be expected. This is reinforced by the rise in respiratory diseases in the past decades. Our genetic sequence has not changed, but the impact of the environment is magnified through the plasticity of our epigenomes. Variable methylation associated with reduced lung function may result from differences in past environmental exposures (either indirectly as biomarkers of exposure or on the casual pathway); the effect of genetic sequence variants that themselves are associated with low lung function; or as a consequence of disease processes such as inflammation (11). Given these potential relationships among methylation, lung function (LF), and disease, interpretation of the

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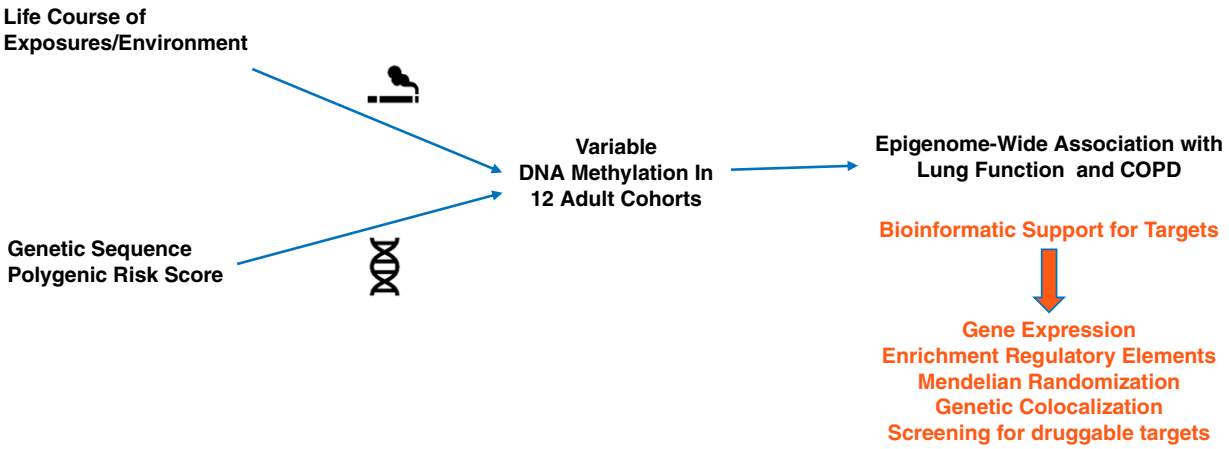

Figure 1. DNA methylation associations with lung function represent a snapshot of a life course of plasticity related to environmental exposures and the impact of genetics. Previous small-scale DNA methylation studies of lung function have linked methylation variation with pulmonary disease. The current multiethnic study integrates methylation with polygenic risk and leverages bioinformatic approaches to highlight functional and potential therapeutic relevance of the epigenome for complex lung diseases. COPD = chronic obstructive lung disease.

results of EWAS studies is difficult. Lee and colleagues have integrated the multitude of approaches to move epigenetic investigation of complex lung disease toward functional relevance.

One important feature of DNAm is that patterns of methylation will vary by cell type and tissue of origin. Thus, observed differences in methylation may result from different proportions of specific cell types between those with high and low lung function; small differences in methylation across all cell types; or larger differences in methylation in specific cell types. Lee and colleagues used the eFORGE tool (12) that capitalizes on the availability of methylation from purified cell types to demonstrate that the LF associated CpGs were enriched for active functional elements in blood (as expected) but also in fetal lung, suggesting that these loci may regulate gene expression in lung cell types potentially starting in early life.

Further evaluation was undertaken by correlating CpG methylation and gene expression in blood; assessing the association between genetic variants associated with LF and CpG methylation; and causal modeling using mendelian randomization analysis. Adjustment of the analysis for a polygenic risk score for each lung function parameter demonstrated that a majority of the EWAS associations captured an independent biologic signal.

The study of Lee and colleagues (10) and previous meta-analyses (9) have all demonstrated that analysis of leukocyte DNAm has potential clinical utility. In addition, they have demonstrated potential to provide insights into the underlying biological mechanisms influencing lung function. So what is next? In terms of the role of epigenetics (specifically DNAm) in the development of low lung function, there are some clear research priorities. Although Lee and colleagues demonstrated enrichment of active DNA elements in lung and some overlap with DNAm signals from a previous study of lung tissue (13), the relevance of LF-associated CpG sites identified in blood to regulation of gene expression in specific cell types in the lung requires further exploration. This may be achieved through lung tissue investigation and extension to single-cell methylation to capture cell types and temporal and spatial aspects of the epigenome at the complex interface of the lung and environment. Furthermore, the arrays used in EWAS studies to date capture <2% of CpG sites in the genome and sequencing-based approaches are needed to fully understand the epigenetic landscape in relation to lung function. Given the strong signature of tobacco smoke exposure, as evidenced by attenuation of the EWAS signals with adjustment for smoking-associated methylation marks and evidence for unique CpG associations in lifetime never-smokers, larger studies are required to understand what proportion of signals are driven by smoke exposure versus other exposures such as air pollution that might affect the same pathways. In addition, it is unclear whether the ancestry differences in LF-associated methylation observed by Lee and colleagues are driven by genetic differences or by differences in exogenous/endogenous exposures. These are fundamental epidemiologic questions that must inform the next wave of epigenetic investigations. A key unanswered question is one of timing: Do these methylation sites predict future lung function decline, or are they representative of lung function growth in childhood and/or *in utero* programming?

There are an increasing number of omics that have been studied to understand the factors associated with low lung function at the population level. What is needed now is integration—bringing together multiple omics approaches in both lung and blood to understand the pathophysiological processes underlying growth and decline of lung function and the heritable, environmental, and pathological triggers of disease. Such integration of omics data, particularly epigenetic and gene expression data, has been particularly valuable in providing insights into the pathogenesis of a range of acute and chronic diseases including atopic dermatitis (14), coronavirus disease (COVID-19) (15) and COPD (16). Network medicine-approaches may hold the most compelling promise to move DNAm insights into clinical translation (17). The study by Lee and colleagues is a major advance in framing the field of pulmonary epigenetics for prognostic, diagnostic, and therapeutic insights and sets the stage for 21st century insights into complex lung diseases. ■

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Biomarkers for Interstitial Lung Abnormalities: A Stepping-stone Toward Idiopathic Pulmonary Fibrosis Prevention?

Interstitial lung abnormality (ILA), defined broadly as the presence of nondependent radiographic abnormalities on computed tomography (CT) scan occurring in an individual in whom interstitial lung disease is not suspected, appears to be a precursor to idiopathic pulmonary fibrosis (IPF) and other forms of progressive pulmonary fibrosis (PPF) (1). ILAs are frequently found in asymptomatic individuals with a strong family history of pulmonary fibrosis (2, 3). In the nonfamilial setting, ILAs are more common with advancing age, in those with the rs35705950 MUC5B polymorphism, and occur in 4–9% of smokers and 2–7% of nonsmokers over the age of 60 (4). Almost half of ILAs progress over the subsequent 5 years, and risk of mortality for those with ILAs is considerably higher than for age-matched populations (5).

Given the significant morbidity and mortality associated with IPF and PFF (6), the identification of individuals prior to the development of irreversible fibrosis and onset of symptoms affords a window of opportunity for genuinely disease-modifying therapeutic intervention. Understanding of the natural history of ILAs has come a

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