Not only does this study suggest the need for increased therapies targeting asymptomatic lung disease in infants and preschoolers, but it also adds to accumulating data that suggest that MRI is a viable outcome measure for future interventional studies of treatment of mild or asymptomatic lung disease. Although sedation with choral hydrate is routinely used for imaging procedures in Europe, advances are being made in MR image acquisition time (14) and distraction techniques (15) to improve imaging results in unsedated infants. The incorporation of such measures may negate the need for sedation in future studies, further expanding the appeal of MRI for very young children.

The landscape of CF therapeutics is changing rapidly, and outcome measures used to detect disease must follow suit. With this well-designed observational study of radiographic abnormalities in infants with CF detected by NBS or clinical symptoms, Stahl and colleagues (13) continue to push forward to establish lung MRI as the standard of care for the detection of mild, asymptomatic CF lung disease. ■

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The Epigenomic Landscape: A Cornerstone of Macrophage Phenotype Regulation in the Fibrotic Lung

Macrophages are immunological cells that are present throughout the lungs. Based on the location and subtype of the macrophage, they play different roles during homeostasis or in the setting of injury or repair. Macrophages, although terminally differentiated cells, have high plasticity and respond to environmental stimulus as well as their anatomical location by having different polarization phenotypes (1). The heterogeneity within the macrophage subpopulations of the lung has been highlighted in the setting of chronic lung diseases such as pulmonary fibrosis. Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, nonresolving interstitial lung disease of unknown etiology that is characterized by excessive extracellular matrix deposition, leading to reduced lung compliance and disruption of gas

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exchange, culminating in respiratory failure and ultimately death. Previous work has demonstrated that macrophages isolated from patients with IPF are composed of different subpopulations and have differential gene expression and metabolic profiles when compared with those isolated from control subjects (2). In addition, two studies have demonstrated that elevated blood monocytes result in increased progression of patients with pulmonary fibrosis, implicating monocytes as a possible biomarker of disease severity (3, 4).

Because of the high heterogeneity of macrophage subpopulations, previous work has identified epigenetics as a key controller of macrophage activation (5). The process of epigenetics regulates how stimuli and environmental changes can modify gene expression within specific cells, without changing the underlying DNA sequence. Most epigenetic mechanisms are related to DNA–protein interactions, and there are several different levels of epigenetic control, such as posttranscriptional histone modification, DNA methylation, and noncoding RNA. Previous work has demonstrated DNA methylation changes are associated with regional differences of macrophage subtype within the lung microenvironment (6). The process of aging is associated with altered epigenetic mechanisms of gene regulation (7). In fact, DNA methylation status has been used to predict chronological age in a variety of tissues because, generally, it has been found that these modifications decrease with age. Using a variety of animal models, it is generally well accepted that there is a linkage betweenepigenetic alternation and the aging process; however, to date, there are no studies examining epigenetic modification and gain in macrophages in the setting of IPF.

Although the function and gene expression of macrophage subtypes within the lung during IPF have been examined, the mechanisms regulating these transcriptomic changes have yet to be determined. In this issue of the *Journal*, McErlean and colleagues (pp. 954–966) investigate the role of DNA methylation in altering airway macrophage gene expression during pulmonary fibrosis (8). The authors examine the DNA methylation profile of alveolar macrophages isolated from patients with IPF and control subjects and compared these data with established Blueprint datasets from representative myeloid cells, including monocyte subtypes and *in vitro*–derived macrophages (8). They determine that the majority of the myeloid-specific CpGs (regions of DNA containing cytosine-guanine nucleotides connected by phosphodiester bond) reside in intronic or intergeneic regions and correlate with open chromatin motifs (as determined by DNase-1 hypersensitivity sites). Additionally, they demonstrate that the DNA methylation pattern in alveolar macrophages is distinct from circulating monocytes or *in vitro* differentiated monocyte-derived macrophages. Although this work provides evidence that methylation patterns of alveolar macrophages are distinct from other subtypes, the majority of the data examining ChIP-Seq (chromatin immunoprecipitation sequencing) and histone modifications were analyzed using datasets from Blueprint (including *in vitro* polarized cells) rather than paired samples from the same patient. Additional experimentation using alveolar macrophages and blood monocytes isolated from the same control subject and patient with IPF are needed to confirm these studies.

Because aging is the strongest risk factor for IPF, the authors were interested in investigating the role of aging using their DNA-methylation profiles and an epigenetic "clock" analysis. Previous work has demonstrated that the weighted average of methylation at multiple different CpG sites can be integrated into estimates of chronological age in humans using previous cohorts to identify CpG sites whose methylation levels can be combined to form an age predictor (9). In the current manuscript, the authors used these defined "epigenetic clocks" to measure changes in DNA methylation to estimate the sample donor age. They found a correlation of the DNA methylation profile with the chronological age, but there was no association with accelerated aging in IPF alveolar macrophages compared with control subjects. Interestingly, they did observe some changes in methylation patterns and gene expression related to lipid and glucose metabolism, which suggests a possible role for epigenetics in regulating the metabolic phenotypes observed in alveolar macrophages from patients with IPF compared with control subjects. Future studies are needed to define whether described changes in DNA methylation patterns translate to changes in protein expression and/or functional outcomes. Overall, these data provide strong evidence that heritable DNA methylation profiles contribute to the altered phenotype of alveolar macrophages in the setting of pulmonary fibrosis.

The strength of this manuscript is the in-depth approach to examine DNA methylation profiles specifically in alveolar macrophages isolated from patients with IPF. Previous work has highlighted altered DNA methylation profiles in patients with IPF compared with control subjects, but these have only been completed using whole lung tissue (10) or isolated fibroblasts (11). In addition, this study examines the role of DNA methylation in regulating an immune cell population. Although resident alveolar macrophages are known to be long-lived cells, the majority of age-related questions have been completed in murine models, and it has been difficult to translate these finding to humans. Taken together, these observations provide a platform for investigating additional methylation-mediated gene expression and function of macrophages in the setting of pulmonary fibrosis.

Although this manuscript highlights some interesting observations, there are some limits to the interpretation of this study because of the use of multiple datasets for completing the analysis. The authors chose to use the EPIC BeadChip Array for their analysis, and although this provides some level of detail regarding the CpG methylation, the data processing can be more challenging and can result in higher background to noise. In addition, the use of CpG methylation as a marker of epigenetic regulation without the use of ChIP-, ATAC (Assay for Transposase-Accessible Chromatin)-, or DNaseI-seq results in a skewed database because not all promoters and enhances have CpG islands. Furthermore, it is known that DNA methylation is only one epigenetic change that can regulate gene expression within different cellular subsets. Additional work examining the contributions of posttranscriptional modification and noncoding RNA are needed to further assess the role of epigenetics in regulating IPF.

Overall, these data demonstrate that the epigenetic modification of DNA methylation distinguishes alveolar macrophages isolated from patients with IPF compared with control subjects. This work is especially exciting because it was performed using all human cells in contrast to other studies performed in animal models. This is the first report of DNA methylation changes in IPF macrophages. Alterations in epigenetic modifications could contribute to better diagnosis and therapeutics to treat or prevent pulmonary fibrosis. Previous studies have measured epigenetic changes in macrophages across multiple

different disease states, including atherosclerosis, obesity, diabetes, and sepsis (5). These results provide important insights for potential epigenetic therapeutics for IPF treatment. ■

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Using Automated Radiographic Signatures to Prognosticate Chronic Lung Allograft Dysfunction

What Does the Future Hold?

Lung transplantation is a life-saving procedure that is associated with a significant improvement in health-related quality of life and physical function (1, 2). However, at 6.2 years, the median survival of lung transplant recipients worldwide lags behind other solid organ transplant recipients (3). The main contributor to decreased survival is chronic lung allograft dysfunction (CLAD), with about half of transplant recipients developing CLAD within the first 5 years (4, 5). Unfortunately, once CLAD develops, the prognosis is poor, with ongoing loss of function in most patients (5). Thus, early identification of graft injury at the time of potential CLAD, represented by an initial drop of 10–20% from baseline FEV_1, may allow for treatment strategies that may help mitigate graft loss and, potentially, reduce morbidity (5, 6). Presently, there is no effective treatment for CLAD other than retransplantation (5, 7).

The recent International Society of Heart and Lung Transplant consensus statement recommends a high-resolution computed tomography (HRCT) evaluation at the time of potential CLAD (5). Although HRCT is most helpful for excluding non-CLAD causes of lung function decline, the systematic use of HRCT at baseline and at CLAD onset can facilitate the identification of imaging biomarkers for earlier discovery of graft dysfunction, CLAD phenotyping, and prognosis. A promising quantitative approach described by Belloli and colleagues (pp. 967–976) in this issue of the *Journal* is parametric response mapping (PRM) (8). PRM is a voxel-wise analysis of paired HRCT inspiratory and expiratory images to identify both air trapping and parenchymal lung diseases, some of which may not be detectable

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