Radiographic Honeycombing and Altered Lung Microbiota in Patients with Idiopathic Pulmonary Fibrosis

To the Editor:

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrotic disease of the lung with increasing incidence and poorly understood pathogenesis (1). The lung microbiome has recently been implicated in IPF pathogenesis, with studies showing that lung microbiota are altered in disease, predictive of disease progression, correlated with variation in alveolar cytokines, and causally involved in animal models (2, 3). Although the radiographic features of IPF have critical diagnostic and prognostic significance (1, 4), the relationship between radiographic findings and lung microbiota is undetermined. To determine the relationship between lung microbiota and the anatomic features of IPF, we compared microbiota in IPF BAL fluid (BALF) with IPF radiographic features. Specifically, we compared microbiota in patients with IPF with and without radiographic evidence of honeycombing detected via high-resolution computed tomography (CT). Honeycombing is a cardinal histopathologic feature of IPF; it increases the diagnostic certainty and independently predicts mortality (4).

To examine lung microbiota and radiographic honeycombing, we studied patients with IPF who were enrolled in the COMET (Correlating Outcomes with Biochemical Markers to Estimate Time to Progression in Idiopathic Pulmonary Fibrosis) study. Bacterial communities in BALF were characterized using 16S rRNA gene sequencing and quantified as previously described (2). Honeycombing was recorded as absent or present on baseline chest high-resolution CT by a thoracic radiologist. Differences in community composition were determined by model-based analysis of multivariate abundance data (*mvabund*) and by multivariate ANOVA with permutation testing. Differences in bacterial DNA burden and diversity were determined using Student's *t* test. A total of 68 patients with data available for analysis were included. The clinical characteristics and demographics of the subjects have previously been described (2), and differences between patients with and without radiographic honeycombing are reported in Table 1. This was a subanalysis of previously published COMET data. The sequencing data are available via the National Center for Biotechnology Information Sequence Read Archive (accession numbers PRJNA515255 and PRJNA515279). Operational taxonomic unit (OTU), taxonomy, and metadata tables are available for download at https://github.com/dicksonlunglab/murine_pulmonary_fibrosis.

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In this hypothesis-generating study, to determine whether the composition of lung bacteria differs among patients with IPF and honeycombing, we first used principal component analysis to visualize the relative similarity or dissimilarity of specimens when clustered by honeycombing status (Figure 1A). Although we observed a considerable overlap in the lung communities of patients with and without honeycombing, we also observed a clustered separation of specimens by honeycombing status. When we compared the communities statistically using *mvabund*, this collective difference in community composition was significant, robust to the taxonomic level of comparison (e.g., $P = 0.006$ at the OTU level of taxonomy, and $P = 0.037$ at the family level). The difference did not meet statistical significance when the communities were compared via multivariate ANOVA with permutation testing ($P = 0.07$, adjusted for age, baseline D_{LCO}, and smoking status). We thus determined that lung communities in patients with IPF and honeycombing are collectively different from those in patients with IPF and no honeycombing, although a considerable taxonomic overlap exists.

To better understand these collective differences in community composition, we next used complementary techniques to compare specific bacterial taxa. We used BiPlot analysis (Figure 1B) to determine which bacterial taxa could explain the separation of specimens in our principal component analysis plot. This revealed several candidate taxa that explained variation in specimens toward the honeycombing-present cluster (e.g., OTU1464 *Porphyromonas* and OTU1428 *Gemella*), as well as several that explained variation toward the honeycombing-absent cluster (e.g., OTU1373 *Cronobacter*). We next used rank abundance analysis to visualize differences in the relative abundance of prominent taxa across groups (Figure 1C). Although the most abundant taxa were common across groups (e.g., OTU1461 *Prevotella* and OTU1463 *Veillonella*), several prominent taxa differed visibly across the comparison (e.g., OTU1464 *Porphyromonas*, OTU1373 *Cronobacter*, and OTU1428 *Gemella*). We directly compared the relative abundance of candidate taxa identified by BiPlot and rank

Table 1. Demographics and Clinical Characteristics of the COMET Idiopathic Pulmonary Fibrosis Cohort

	No Honeycombing	Honeycombing	*P* Value
n (%)	29 (43%)	39 (57%)	—
Age, yr, mean ± SD	62.7 ± 7.3	66.5 ± 7.0	0.03
Male, *n* (%)	20 (68.9%)	26 (66.6%)	0.99
FVC% predicted, mean ± SD	72.0 ± 16.6	69.8 ± 18.3	0.61
D_{LCO}% predicted, mean ± SD	49.3 ± 13.1	39.4 ± 13.6	0.007
Smoking status			0.006
Non, *n* (%)	14 (48%)	6 (15%)	—
Ever, *n* (%)	15 (52%)	33 (85%)	—

Definition of abbreviation: COMET = Correlating Outcomes with Biochemical Markers to Estimate Time to Progression in Idiopathic Pulmonary Fibrosis. ANOVA, unpaired *t* test, or Fisher's exact test was used when applicable.

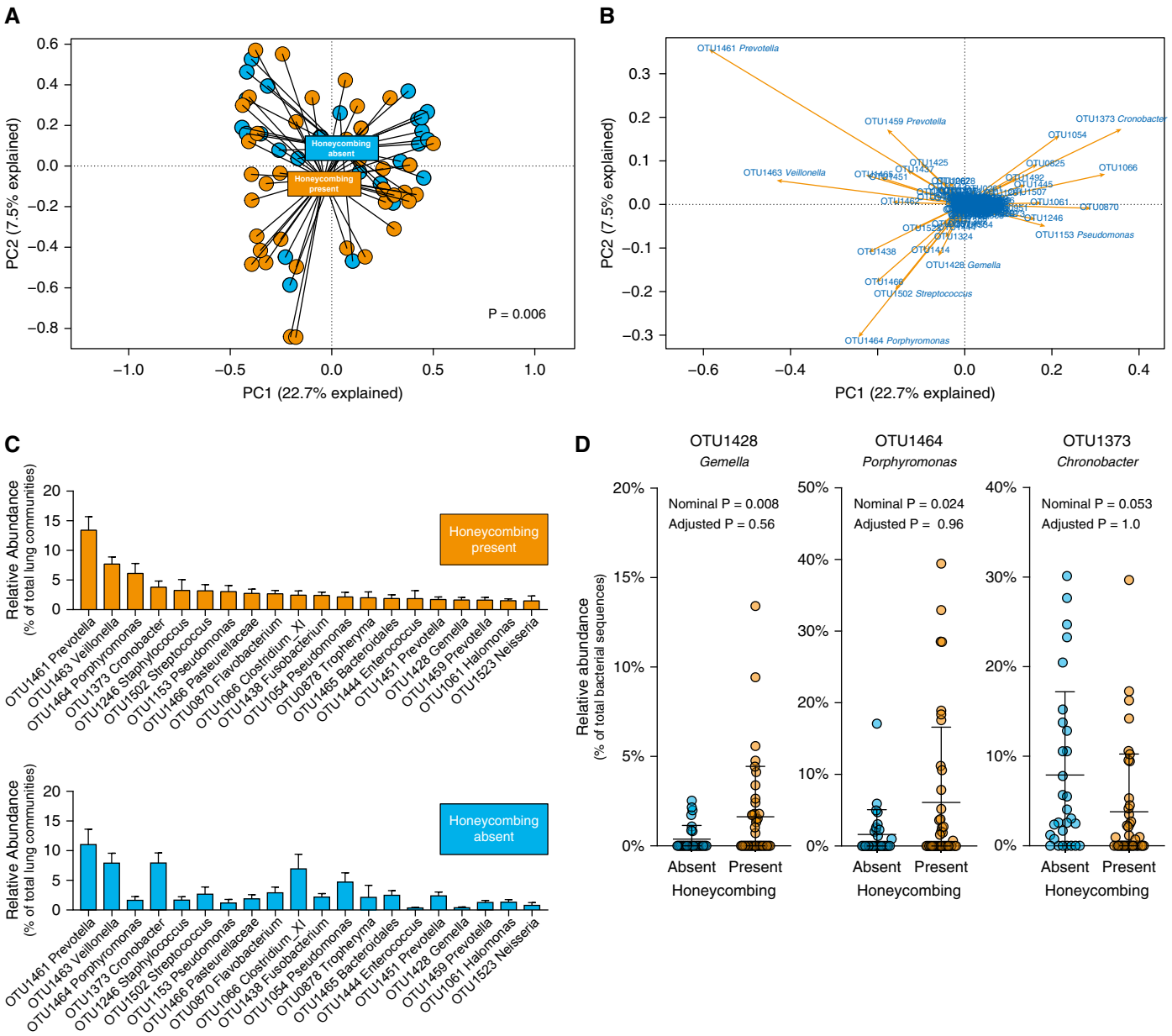

Figure 1. In patients with idiopathic pulmonary fibrosis, radiographic honeycombing is associated with changes in lung microbiota. Sixty-eight patients with idiopathic pulmonary fibrosis were stratified by the presence or absence of honeycombing on computed tomography scan. Lung microbiota were analyzed using amplicon sequencing of the 16S rRNA gene. Operational taxonomic unit (OTU) data in the principal component (PC) analysis were Hellinger transformed to allow for better behaviors with longer species gradients. (*A*) Ordination of specimens by community composition (principal component analysis) revealed clustering by honeycombing status, although considerable overlap was observed. Overall community composition was significantly distinct across groups ($P = 0.006$, *mvabund*). (*B*) BiPlot analysis of this ordination identified candidate bacterial taxa responsible for driving the separation of honeycombing specimens (e.g., OTU1428 *Gemella* and OTU1464 *Porphyromonas*). (*C*) Rank abundance analysis revealed taxonomic differences between groups. The 20 most abundant taxa in honeycombing-present specimens are shown in decreasing order of mean relative abundance (mean ± SEM). (*D*) Specific bacterial taxa were then compared across groups using direct comparison of mean relative abundance. Although select taxa were nominally significant when compared directly (e.g., OTU1428 *Gemella* and OTU1373 *Chronobacter*), no specific taxa were significant when adjusted for multiple comparisons by *mvabund*. Significance was determined via *mvabund*, and the nominal *P* values presented were obtained before adjustment for multiple comparisons (*A* and *D*).

abundance analysis (Figure 1D). We found that although OTU1428 *Gemella* and OTU1464 *Porphyomonas* were nominally enriched in specimens from patients with honeycombing ($P = 0.008$ and 0.024, respectively; *mvabund*), these comparisons were not significant when controlled for multiple comparisons ($P > 0.05$ for both). Similarly, although OTU1373 *Cronobacter* was nominally enriched among honeycombing-absent specimens, it was not significantly

enriched when adjusted for multiple comparisons ($P > 0.05$). *Cronobacter* OTU1373 was detectable at a low signal (2.63% of reads) in negative controls. Both *Gemella* OTU1428 and *Porphyromonas* OTU1464 were not detectable in negative controls. We then compared the burden of bacterial DNA and the diversity of bacterial communities (Shannon diversity index) in BALF from patients with IPF with and without honeycombing. We found no significant difference in either feature of the microbiome ($P > 0.05$). We concluded that although honeycombing alters community composition, it is not associated with differences in bacterial burden or diversity.

Here, we demonstrate that the honeycombed lung is associated with alterations in the community composition of lung microbiota. These taxonomic differences are appreciable at both the species/genus and family levels of taxonomy and may not be directly attributable to individual taxa but rather to collective differences in community composition. Several studies have demonstrated the importance of lung dysbiosis in IPF. Molyneaux and colleagues have shown that a greater bacterial burden is predictive of increased mortality (5), a finding we recently validated (2). Studies have linked changes in the expression of host defense genes in blood with lung microbiota in IPF (3). Immunosuppression has a deleterious effect on clinical outcomes in patients with IPF, although antibiotic therapy in IPF may convey a clinical benefit (6, 7). A recent study reported minimal bacterial signal in excised lung tissue from patients with IPF (8), which may suggest that the host–microbiota interface can be more readily sampled via the airway mucosa (BALF) than from peripheral parenchymal tissue, which is largely comprised of extracellular matrix.

Honeycombing is a feature of IPF with pathologic and prognostic significance. It is believed to derive from mucin dysfunction in the distal airway, whereby epithelial cells exhibit increased expression of Muc5b, a mucin that is essential for normal mucocillary clearance (9). Overexpression of mucin in the distal airway may alter the ecological conditions of the lungs, selectively promoting dysbiosis. We observed a potential association between honeycombing and a member of the *Gemella* genus. *Gemella* belong to the Firmicutes phylum, are found at the mucosal surface of the aerodigestive tract, and have been implicated in exacerbations of chronic lung disease (10). We speculate that honeycombing results in ecological changes to the distal airways, fostering the growth of taxa such as *Gemella* spp., altering community composition, and contributing to injury from mucin overexpression and defective mucocillary clearance.

Our work has several limitations. We were unable to examine the topographical extent and severity of honeycombing in our study cohort. BALF was acquired from the right middle lobe or lingular segment based on the extent of comparative disease between these two locations, but the severity of honeycombing was not recorded or incorporated into our analysis. It may be that graded relationships exist between honeycombing and lung microbiota. Furthermore, interobserver variability exists in the reporting of CT honeycombing. It is possible that confounding is present in lung microbiome studies (e.g., due to medications or disease severity) that cannot be known or accounted for in our analysis. The size of our study cohort and the power to detect differences were limited. Therefore, these observations should be further examined in other cohorts of patients with IPF.

Our exploratory findings suggest a bidirectional interaction between lung microbiota and the anatomic disruption of IPF. Future studies should interrogate further the causal role of dysbiosis in the progressive tissue distortion and honeycombing of IPF to determine whether the lung microbiome represents a therapeutic target in IPF. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Effect of Nusinersen on Respiratory Muscle Function in Different Subtypes of Type 1 Spinal Muscular Atrophy

To the Editor:

Spinal muscular atrophy (SMA) is the leading inherited cause of infant death. However, the rapidly evolving landscape of therapeutic options is dramatically changing the natural history of the disease. Nusinersen was the first drug to be approved for the treatment of SMA, and it is now commercially available in many countries worldwide. Very recently, the gene therapy onasemnogene abeparvovec-xioi also received approval from the U.S. Food and Drug Administration for the treatment of patients younger than 2 years of age. Nusinersen is administered intrathecally and consists of an antisense oligonucleotide designed to modify pre-mRNA, thus increasing the level of SMN protein (1). A phase 3 randomized, double-blind, sham-controlled, clinical trial in patients with SMA1, the most severe form of SMA, showed that patients treated with nusinersen had a significant motor milestone response and a higher likelihood of event-free survival (2) (i.e., free from tracheostomy or noninvasive permanent ventilation). However, there is no information regarding the effects of the treatment on respiratory function. Such effects can strongly affect the prognosis in SMA1 because respiratory problems are the major cause of hospitalization, morbidity, and mortality. Ribcage muscle weakness is a distinctive feature of SMA1 since infancy, whereas the diaphragm is spared and carries out the breathing function (3), and natural history studies have shown that the majority of surviving children with SMA1 become ventilator dependent (4, 5). The assessment of respiratory function in infants is challenging because it usually requires invasive and/or volitional tests (6). We have previously reported how feasible and informative noninvasive measurements of the respiratory pattern during quiet breathing by optoelectronic plethysmography can be, even in uncooperative infants. We have characterized the specific ventilatory and thoracoabdominal patterns that occur during quiet breathing of infants and children affected by the three forms of SMA. Specifically, patients with SMA1 were characterized by rapid and shallow breathing with paradoxical thoracoabdominal motion (3) and a bell-shaped thorax (7). The study was performed in 32 untreated patients with SMA1, who might be considered a control group of natural-history patients. In the present study, respiratory function data from the control group were compared with those obtained in a new cohort of 27 infants and children with SMA1 who were treated with nusinersen. For the very first time, we were able to determine whether and how nusinersen affects respiratory function in the most severe form of the disease.

To better capture possible responses to treatment, which have been reported to be variable depending on different factors, including age at treatment and disease duration and severity, we decided to divide both the control subjects and the treated patients with SMA1 into three subtypes, defined

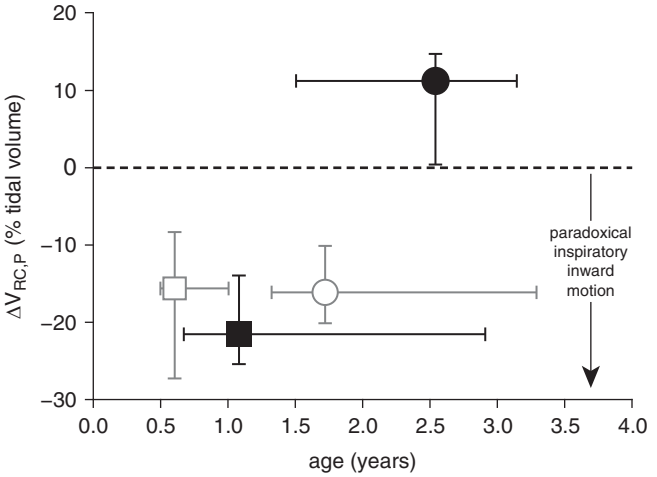

Figure 1. Median (symbols) and interquartile range (whiskers) of age (*x*-axis) and percentage contribution of the pulmonary ribcage to V_T ($\Delta V_{RC,P}$; *y*-axis) in children with spinal muscular atrophy 1 subtypes A and B (open grey symbols) and C (solid black symbols), treated (circles) or not (squares) with nusinersen. Negative values indicate the presence of paradoxical inspiratory inward motion of the pulmonary ribcage.

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