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Oral Microbiota and Pediatric Asthma Phenotype A New Window for Biomarkers?

The ongoing burden of asthma globally (1, 2) necessitates continued investigation into the mechanisms that underlie asthma development and different clinical trajectories. Furthermore, it is known from longitudinal studies that childhood asthma is an important risk factor for the development of chronic obstructive pulmonary disease in adulthood, highlighting the importance of early-life events that shape lung development and function (3). The microbiome is a critical interface between exogenous exposures and immune development in early life. The gut microbiome has been studied the most intensely, but the microbial community of the airways is also believed to be an important component in the multifactorial underpinnings of childhood asthma (4–8). Although the upper airway nasopharyngeal microbiota has been associated with asthma characteristics in children, sampling the lower airways is more difficult. Moreover, an underlying question is whether the oropharyngeal microbiota could be informative of asthma pathophysiology and could play a role in pediatric asthma phenotype, including preschool wheezing and exacerbations.

In this issue of the *Journal*, Abdel-Aziz and colleagues (pp. 142–154) address this question by analyzing the microbiota of

oropharyngeal swabs collected from 241 children recruited with mild, moderate, or severe school-age asthma or preschool wheezing as part of a multicenter European observational cohort study (U-BIOPRED [Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes]) (9). The authors hypothesized that although no differences in microbial diversity were previously found to be associated with disease severity in this dataset (10), there could be compositional differences that correlate with clinically relevant phenotypes.

Oropharyngeal swabs were processed for 16S ribosomal RNA target gene sequencing, and the data were used to compare bacterial composition across samples (β -diversity). The samples clustered into four distinct microbial communities that displayed different within-sample diversity (α -diversity) and that were each enriched with one or two of the five most abundant bacterial genera—*Streptococcus*, *Veillonella*, *Haemophilus*, *Prevotella*, and *Rothia*—often considered potential pathobionts (11).

Interestingly, the bacterial community profiles themselves were associated with distinct clinical characteristics, such as atopic dermatitis diagnosis, spirometry, and atopic sensitization to grass pollens. For example, Cluster 1, dominated by *Streptococcus*, was associated with the lowest percentage of atopic dermatitis and atopic sensitization to grass pollen. Furthermore, the clusters identified at baseline were predictive of annual asthma exacerbation frequency during a 12–18-month follow-up (Figure 1). For example, children who fell into the *Veillonella*-dominant Cluster 2 had a significantly higher odds ratio of having two or more exacerbations per year as compared with children with microbial profiles enriched with taxa of the genera *Rothia* (Cluster 3) or *Haemophilus/Neisseria* (Cluster 4). This type of observation points to a role these taxa may play in the pathogenesis of asthma and pediatric phenotypes.

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E.G. is supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases. Y.J.H. is supported by National Institute of Allergy and Infectious Diseases grant R01AI129958.

Originally Published in Press as DOI: 10.1164/rccm.202305-0856ED on June 5, 2023

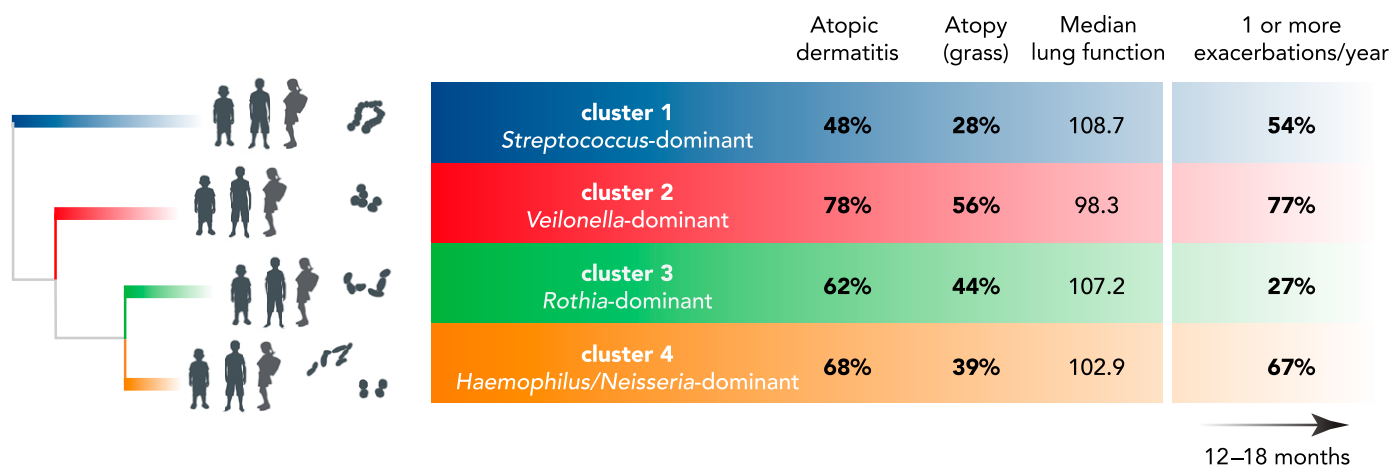


Figure 1. Percentage of children within taxa-driven clusters that have associated clinical characteristics and future exacerbations during 12–18-month follow-up. As per Abdel-Aziz and colleagues (9), preschool- and school-age children with wheezing or asthma were stratified on the basis of their oropharyngeal microbial profiles dominated by specific taxa. Lung function test: FEV₁ percent predicted after salbutamol; value listed represents the median.

In a complementary analysis, the authors looked at systemic gene expression profiles by microarray analysis of peripheral blood RNA. They identified two hallmark pathways that were statistically different across the clusters after multiple testing corrections: the Wnt/ β -catenin pathway associated with Cluster 4 and the transforming growth factor- β signaling pathway associated with Cluster 2. Both pathways have been reported to be involved in airway remodeling in atopic asthma (12, 13).

A key finding of this study is that clustering children with school-age asthma and preschool-age wheezing by their oropharyngeal microbial profile revealed distinct clinical characteristics between the clusters, such as atopic dermatitis and grass pollen sensitization, as well as future exacerbations. This noninvasive sampling followed by an unbiased clustering approach offered comparatively more insight into oral microbiota patterns related to distinguishing characteristics of pediatric asthma (and potential differences in underlying mechanisms) than comparing the microbiota patterns across predefined clinical strata of asthma severity, as had previously been attempted (10). The authors suggest that the interaction between oropharyngeal microbiota and allergen could play an outsized role in the pathogenesis of childhood asthma and should be further explored.

Although the new insights provided by this study could inform better diagnostic and management strategies, there are a number of limitations. One obvious limitation of this study is that the participants are largely (80%) White children. Determining whether similar taxa-driven clustering of phenotypes in more ethnically diverse populations would be of crucial importance for translational purposes. Critical information about preceding antibiotic exposures, perinatally and up to the time of sample collection, was not available, which could impact the findings. Similarly, corticosteroids can alter the microbiome, and 88% of the cohort were receiving inhaled corticosteroid treatment. Adjusting for this exposure in the statistical analyses may not be sufficient to dissect the impact of chronic medications on a microbiome. Other limitations of this study, as the authors also acknowledge, are that the taxonomic assignments are

limited largely to genus-level designations, limiting insight on which bacterial species could be targeted. Similarly, 16S rRNA data limit functional assignments. Defining microbial pathways that contribute to disease pathogenesis could lead to better, microbially informed therapeutic strategies. Function-oriented assessments of the microbiome, like metagenomics, are expensive and more so for airway studies because of the lower microbial biomass compared with the gut. For more mechanistic insights, metatranscriptomics would be more powerful. Input requirements may be difficult from clinical specimens with limited starting material. However, if successfully performed, one advantage is that the host RNA sequence reads could be analyzed separately from the microbial RNA profile. Such data would help compensate for limitations of blood gene expression and would look at *in situ* functional profiles. They would also capture expression profiles of other microbes, such as fungi as well as DNA and RNA viruses, that could be involved in exacerbations. As such, the study also did not take into account viral triggers of wheezing and asthma symptoms, which could be better predictors of future exacerbations (14).

In summary, this study demonstrates the potential utility of analyzing the oropharyngeal microbiota to define bacterial profiles associated with asthma clinical phenotypes, and not necessarily disease severity, in children with asthma or preschool wheezing. Oropharyngeal sampling is noninvasive and relatively inexpensive, so it is an attractive consideration. The findings reported by Abdel-Aziz and colleagues generate a number of new hypotheses for which further research is needed to understand the mechanisms by which the oral microbiota may influence clinical trajectories of asthma. ■

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

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Finding the Right Biological: Eosinophil Subset Differences in Asthma and Chronic Obstructive Pulmonary Disease

Asthma practice guidelines recommend considering eosinophil counts from blood, induced sputum, or both for treatment decisions (1). However, controversy persists on the relevance of eosinophils to chronic obstructive pulmonary disease (COPD) outcomes. Association of eosinophils with greater risk of COPD exacerbations has been either claimed for blood (2, 3) or refuted for both blood and sputum (4). Although 15–37% of patients with COPD persistently have >2% blood eosinophils (5), low rather than high blood eosinophils were associated with both greater emphysema and lung function decline in Global Initiative for Chronic Obstructive Lung Disease group D disease (6). A recent systematic review concluded that blood eosinophil counts cannot predict COPD clinical outcomes (7).

Studies of lung samples also do not support a clearly deleterious role of eosinophils in COPD. Numbers of eosinophils in lungs resected from smokers did not differ in those patients without versus with COPD (8). Eosinophils in BAL were significantly increased in current (but not former) smokers with COPD relative to ever-smokers without COPD or never-smokers (9).

However, because these studies assayed eosinophils as a homogeneous cell type, the recognition of eosinophil subsets (10) necessitates their reevaluation. In both mice and humans, eosinophils exist either as inflammatory eosinophils (iEos) having high expression of the IL-5 receptor α-chain (IL-5Rα, CD125) or as tissue-resident eosinophils (rEos), which have regulatory properties (11, 12). Distinguishable by surface phenotype, these subsets differ in developmental pathways, anatomic localization, and roles (10).

In this issue of the *Journal*, Cabrera López and colleagues (pp. 155–162) examined phenotypes and proportions of these eosinophil subsets in peripheral blood in four discovery cohorts ($n = 10$ each): participants with allergic asthma not receiving biologicals, ever smokers with or without COPD, and healthy volunteers (13). Using a published flow cytometric approach (11), they distinguished iEos (Siglec-8⁺, CD62L^{low}, CD123 [IL-3 receptor]^{high}) from an alternative phenotype (Siglec-8⁺, CD62L^{high}, CD123^{low}) termed rEos, although detected in blood, for consistency with previous literature. Their results showed significantly higher iEos proportions in participants with asthma than in participants

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Supported by the Clinical Science Research and Development Service, U.S. Department of Veterans Affairs Merit Review Award I01 CX000911 (C.M.F.) and the NIH grants R01 HL144718, U01 HL137880 (C.M.F., J.L.C., A.T.H.), and R01 HL144849 (J.L.C.). The contents do not represent the views of the U.S. Department of Veterans Affairs or the U.S. government.

Originally Published in Press as DOI: 10.1164/rccm.202305-0811ED on June 13, 2023