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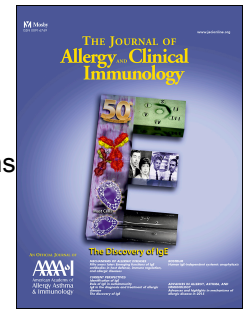

Microbiome in Upper Airway Disease: Moving from taxonomic findings to mechanisms and causality

Vijay R. Ramakrishnan, MD, Daniel N. Frank, PhD

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Journal of Allergy and Clinical Immunology *Invited Editorial*

Microbiome in Upper Airway Disease:

Moving from taxonomic findings to mechanisms and causality

Vijay R. Ramakrishnan, MD[1] and Daniel N. Frank, PhD[2,3]

Department of Otolaryngology[1],
Division of Infectious Diseases[2],
and the Microbiome Research Consortium[3],
University of Colorado School of Medicine, Denver CO

Corresponding author:
Vijay R. Ramakrishnan, MD
Department of Otolaryngology, University of Colorado
12631 E 17th Ave, B205
Aurora, CO. 80045
Phone: 303-724-1965
Fax: 303-724-1961
E-mail: Vijay.Ramakrishnan@ucdenver.edu

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Bacterial and viral pathogens have long been implicated in rhinitis and chronic rhinosinusitis (CRS), and with other atopic diseases such as asthma and atopic dermatitis. In parallel with the evolution of microbiome research methods, interest in airway microbiology has broadened to include not only pathogens, but commensal organisms. The concept of the community as pathogen [1] is likely to be important in CRS, and potentially many other disorders, wherein community-wide microbial function may be pathogenic rather than overgrowth of virulent species. Discovery of the gut microbiome's role in mucosal and systemic immunity has prompted consideration of the relevance of airway microbiota to local mucosal immune function. Numerous microbiota alterations ("dysbiosis") have been implicated in both airway and atopic diseases, although findings have not been universally consistent, and have yet to include evaluation of the virome despite the importance of viruses in the development of childhood respiratory diseases. To date, many of these studies have used cross-sectional observational study designs without assessment of the host response to microbiome alterations, thus limiting our ability to distinguish cause from effect in linking dysbiosis with any particular disease [2].

Establishment of the microbiome early in life is a subject of intense research, and many factors—including antibiotics, birth mode, diet, and genetics—shape this dynamic process. Ultimately, distinct climax communities are established across all body sites exposed to the environment. Understanding the factors driving colonization is important, because both early and late microbial colonizers are likely to have significant effects on host physiology, especially with regards to development of immunological and metabolic homeostasis [3]. For example, in both animal models and human observation, gut microbiome alterations are not only associated with atopic disease, but changes in the functional capacity of gut microbiota result in pro-inflammatory sequelae leading to airway inflammation and hyper-reactivity. However tempting,

we should be skeptical that properties governing the gut microbiome must necessarily apply to the airways.

In this month's JACI issue, Huy Ta and colleagues [4] used 16S rRNA gene sequencing (<150 bp of the V3V6 region) to monitor development of nasal cavity microbiota over the first 18 months of life to predict the onset of rhinitis and early wheeze. This longitudinal case-control study evaluated infants in the GUSTO birth cohort study who subsequently developed rhinitis and wheeze. Enrollees had serial anterior nasal cavity swabs taken over the first 18 months of life, and those who subsequently developed rhinitis with or without wheeze were compared to healthy controls. Overall bacterial diversity was not only lower in both rhinitis groups compared to controls, but also decreased over time, whereas healthy subjects' diversity *increased* with time. Initially, subjects clustered separately by disease state, with increased *Corynebacterium spp.* associated with health and increased Proteobacteria associated with disease. These findings were more extreme in the rhinitis + wheeze group. The reduction in corynebacteria in the disease state is consistent with published data on acute otitis media and wheeze, and the authors drew parallels to the beneficial role of *Corynebacterium accolens* in other airway diseases [5,6].

The authors concluded that because local microbiome changes preceded and developed with disease, their findings "strongly suggest a role of the nasal microbiome in the development of respiratory disease." Leveraging an early life longitudinal birth cohort, this study has begun to move beyond associations into establishment of a real role for the microbiome in disease, especially as many of the findings replicate those in existing literature. However, additional longitudinal research is required to fully understand the role of the microbiome in allergic diseases. Of note, differences in microbiota became less noticeable over time and disappeared by twelve months of age. To further complicate matters, follow-up in the GUSTO cohort indicated

that only 20% of the infants with rhinitis had persistent disease at 5 years. Taken together, these findings suggest the existence of an early window of vulnerability to development of rhinitis and wheeze in which even transient differences in microbiota either contribute to, or at least signify, increased disease risk. The factors governing the dynamics of nasal microbiota, pathogenic mechanisms exerted by the microbiota, connections between the nasal microbiota and lower airways, and why some infants developed persistent rhinitis while many cases resolved remain key knowledge gaps.

Also in this JACI issue, Mahdavinia et al [7], report that corynebacteria were associated with a healthy state in a cross-sectional study of 111 adult CRS patients and 21 non-CRS controls. In this consecutive cohort, middle meatus swabs were obtained endoscopically in the clinic setting, and subjected to sequencing of the bacterial 16S rRNA V4 region. No differences in microbial diversity were reported, but two genera were depleted in the CRS group compared to controls (*Corynebacterium* and *Peptonophilus*), while analysis of the CRS subgroup revealed unique findings in CRS with atopy (decreased *Corynebacterium* in allergy, increased *Streptococcus* in asthma and atopic dermatitis). Particular attention was paid to atopy in this study, which separates it from numerous other cross-sectional CRS studies that have been published to date [8].

A limitation of the case-control study design is that it remains unclear if the microbiome drives the onset, chronicity, or severity of CRS, the presence of CRS initiates changes in the microbiota, or if both are modified by a lurking or confounding factor, such as exposure to tobacco smoke for example (Figure 1). To further investigate this dilemma, the authors used PiCRUST, a software tool that predicts the functional capacities of microbial communities based on 16S rRNA sequence profiles. Applying PiCRUST to their middle meatus bacterial rRNA

sequence datasets, the authors identified two functional pathways unique to the CRS group implicated in pathogenesis – lipopolysaccharide biosynthesis and invasion of epithelial cell pathways. A caveat of these analyses is that functionality is inferred by reference only to existing bacterial genomic sequences, so one wonders what additional genes and non-bacterial taxa would have been identified by direct, shotgun metagenomic sequencing of their specimens. Additionally, bacterial yield in this study was not reported, leaving us to wonder if sufficient bacteria were recovered for use of such predictive analyses, or if sufficient biomass is present in the sinonasal cavity to accomplish such processes on a biologically relevant scale.

Although both of this issue's microbiome studies build on the published literature, expanding in their respective fashions beyond the existing correlative surveys, it is important to note that many questions concerning the role of the microbiome in disease pathogenesis remain. We are all familiar with dictum that "correlation does not equal causation" (ie, *post hoc* fallacy), but the occurrence of microbiome alterations before or alongside the disease state likewise does not prove its importance (ie, *cum hoc* fallacy). A shortcoming of both studies is that they remain observational and associative, like the majority of upper airway microbiome studies to date. Although many interesting hypotheses were generated by the studies, no follow-up experiments were performed. The authors of both studies referenced mechanistic studies of particular species to parallel the taxonomic findings from their respective disease cohorts. For instance, Mahdavinia et al. cite the literature to hypothesize that loss of *Peptinophilus* in CRS may produce unchecked activation of innate lymphoid cells resulting in allergic rhinitis and type 2 inflammatory disease, based on a food allergy study of clostridia-containing microbes [9]. However, some caution must be exercised because microbiome studies that rely on short-read sequencing technologies to generate 16S rRNA gene profiles are, at this time, generally limited

to genus-level taxonomic assignment at best, depending on the variable region(s) sequenced and algorithm used to cluster sequences into operational taxonomic units. Additionally, species-specific and strain-specific functional mechanisms are not necessarily retained in proportion to higher-level taxonomic classification assignments. As such, a mechanistic burden of proof is required to establish that local dysbiosis is a causative factor and institute therapies aimed at microbiota manipulation. This has been absent from most CRS microbiome studies, in part due to a lack of robust animal models.

A number of controversies in the airway microbiome literature serve as a reminder that we are still in the early stages of understanding its role in human atopic diseases. Much more in-depth understanding is required before we condemn antibiotics and Cesarean-sections and recommend healthy donor mucus transplants! The two studies discussed in this editorial move in the right direction by use of a longitudinal birth cohort and predictive functional analytics. Well-defined cohorts, longitudinal sampling, accounting for treatment-associated variables and confounding factors, and further attempts to move beyond associations towards causality are requisite steps to build on these studies.

149 **Figure Legend**
150
151 **Figure 1.** A **limitation of cohort and case-control study designs in microbiome research is**
152 **the inability to disentangle causality, owing to the potential dependency of all variables on**
153 **each other.**
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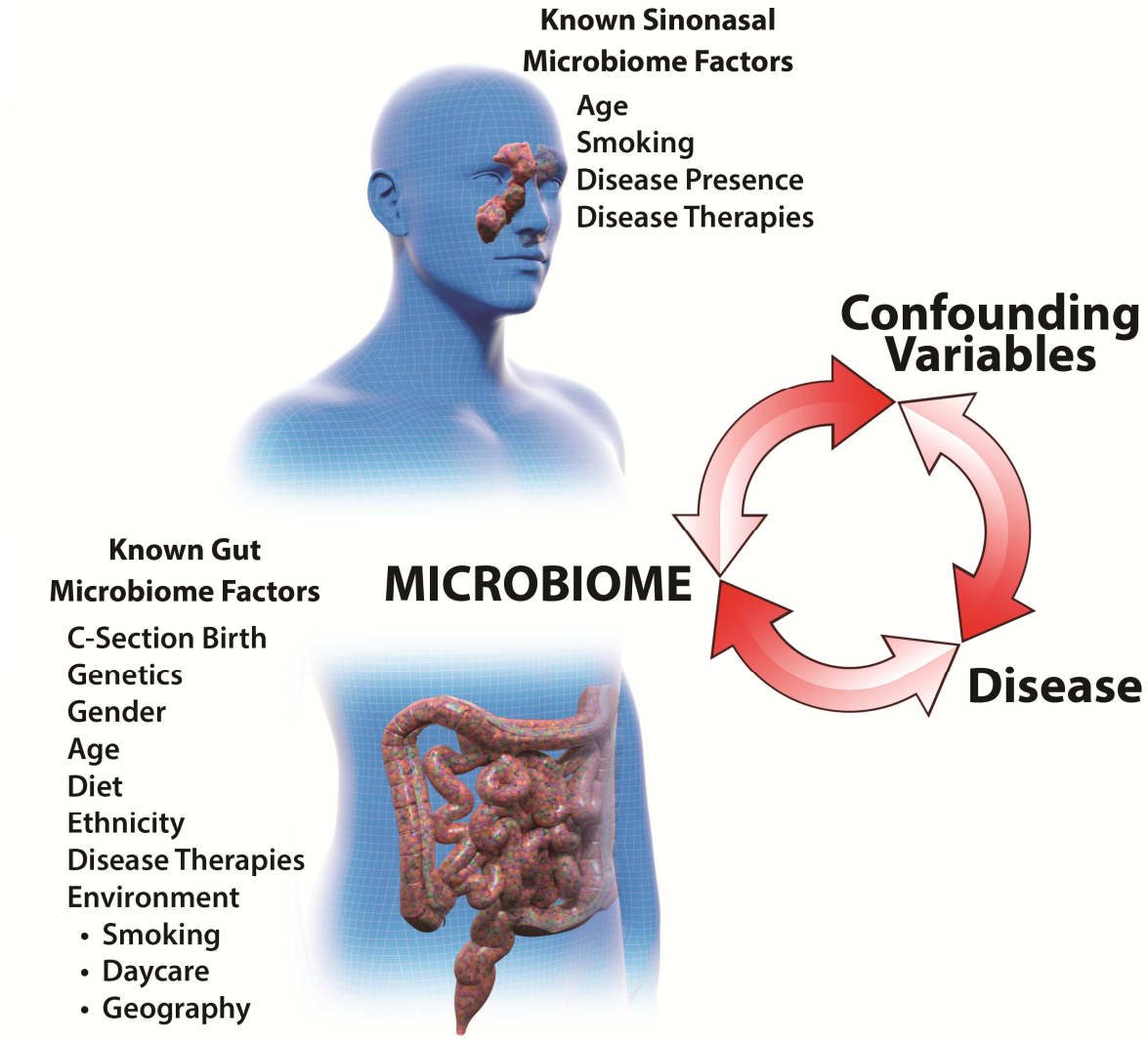
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Microbiome Factors
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Disease Therapies
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• Smoking
• Daycare
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