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Nasopharyngeal Microbiota: Gatekeepers or Fortune Tellers of Susceptibility to Respiratory Tract Infections?

Investigation of the upper respiratory microbiome has drawn increasing, intense interest for several reasons. First, the nasal passages are constantly exposed to the external environment and are the first interface with the respiratory mucosa for many inhaled compounds, particulate matter, allergens, and microbes. Second, the nasopharyngeal (NP) space is relatively accessible for sampling the respiratory tract, allowing for study of larger numbers of subjects by less invasive methods. Third, upper respiratory infections are important clinical events that not only confer significant morbidity but also can affect lung development and increase the risk for asthma in childhood (1). The nasopharynx thus can be viewed as a gatekeeper of respiratory health. However, the extent to which NP microbiota contribute to this gatekeeper role, and the mechanisms by which this may occur, remain unclear.

Recent studies in infants and children have reported important associations between the composition of NP bacterial microbiota and upper respiratory infection outcomes, and in some cases, associations with risk for recurrent wheeze or childhood asthma (2–6). Such studies leveraging sensitive techniques such as 16S ribosomal RNA (rRNA) gene sequencing to identify bacterial communities are representative of the advancement in microbiome investigation from simple characterization studies to *a priori* hypothesis testing of microbiome–disease–phenotype associations. However, a number of issues still hamper studies of the respiratory microbiome in general. One is the interindividual heterogeneity in airway microbiota

composition, both in health and disease, particularly when viewed at finer levels of taxonomic detail (i.e., genus or species level) (6, 7). This means larger subject numbers may be required to reveal microbiome-related differences between groups of interest, and obtaining sufficient samples especially from the lower airways can be a challenge. Second, the stability of the respiratory microbiome remains unclear but is important to establish to interpret associations with disease outcomes; cross-sectional analyses represent the majority of evidence to date, with few exceptions (7–10). Recent studies of the pediatric NP microbiome have begun to prospectively address such issues, as exemplified in the study reported by Bosch and colleagues (pp. 1582–1590) in this issue of the *Journal* (11).

This study reports findings from analyses of the NP bacterial microbiota sampled by swabs from 112 healthy infants in a prospective Dutch birth cohort. A primary goal was to examine trajectories of NP bacterial microbiota development in the first of life that are associated with frequency of respiratory tract infections (RTIs; 0–2, 3–4, or 5–7 RTIs). Frequent sampling was performed by trained professionals in which term infants had NP swabs collected shortly after birth and at days 1, 7, and 14, and then monthly up to 4 months and at 6, 9, and 12 months. NP swabs were also collected at the time of symptomatic RTIs (home visits), defined by the presence of both fever and respiratory symptoms. A total of 1,121 samples were analyzed by 16S rRNA gene sequencing and a multitude of statistical and data visualization techniques. As previously reported from a subset of this cohort, differences in NP bacterial composition were independently associated with mode of delivery and infant feeding (12, 13). Because

age-related changes in NP bacterial composition also were previously observed from this cohort (11), a machine-learning approach was first applied to identify an optimal number of age-discriminatory taxa in their reference group of infants with 0–2 RTIs. From this, a model was developed to apply toward predicting the chronologic age of the microbiota patterns seen in the two groups that experienced more frequent RTIs. This feature of the microbiota patterns, referred to as “microbiota age” by the authors, was found to differ significantly in children experiencing 5–7 or 3–4 RTIs compared with the reference group. Interestingly, the aberrant patterns of NP bacterial composition were apparent very early, with divergence in the frequent RTI groups seen in the 1-month-old samples. The most salient differences in the highest RTI group were a significant underrepresentation of *Corynebacterium* and *Dolosigranulum* (CDG cluster) and earlier appearance and overrepresentation of *Moraxella* taxa compared with the reference group. These observations suggest this pattern of NP bacterial composition is associated with increased susceptibility to symptomatic RTIs in infants.

The intense sampling performed in the first few weeks of life, coupled with sophisticated analytic approaches, enabled these revelations and further hone our attention to the very early life period. These findings converge with the wealth of existing evidence that perinatal, and even prenatal, exposures shape our microbiomes and immune function in critical ways that affect health outcomes down the road. What is novel is the idea that these microbiome-mediated interactions may not be confined to the gut, but might also be occurring locally in the respiratory tract during infant development. A recent Australian study also found that early-life differences in NP bacterial composition (<7 wk of age) was associated with recurrent wheeze or asthma in childhood (4). Such findings raise recognition about the potential importance of the NP microbiome to respiratory health, but many questions remain.

First, what are the functional implications of these different NP microbiota patterns? Assessment of expressed immune functions in the NP mucosa were not reported, which might provide insight into whether aberrant NP microbiota age patterns are linked to different local immune profiles. Moreover, despite analyses to gauge the importance of *Moraxella* in the prediction models, are overrepresented *Moraxella* the main culprit in RTI susceptibility, or is the dramatic underrepresentation of CDG just as important for their putative protective role? The bacterial species and/or strains involved in these relationships also are not certain from 16S rRNA-based analyses. Second, the etiology and timing of RTIs in this study are uncertain from the data shown. No results of viral PCR or bacterial culture studies are reported, which would be of interest given previously reported associations between symptomatic (but not asymptomatic) rhinovirus infections and differences in NP microbiota composition (2, 3). Finally, it is well-established that the gut microbiome is dynamic and actively shaping immune development from birth onward. It is possible that the early divergent NP microbiota pattern associated with frequent RTIs are simply earmarking infants more likely to have RTIs because of differences in developing immune function. Despite these caveats and lingering questions, this is a study to be emulated for its carefully thought out study design, standardization of sample collection methods, and transparency of methodologies that are clearly described. Future studies will better elucidate whether NP microbiota patterns are simply fortune-tellers of individuals at risk for frequent RTIs or other outcomes, or whether the NP microbiome (the totality of microbes, their genomic functions, and interactions within a

niche) serves a critical gatekeeper role in determining risk for respiratory disease. ■

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