Immunological Reviews

WILEY

Immunological Reviews

INVITED REVIEW OPEN ACCESS

The Microbiome in Asthma Heterogeneity: The Role of Multi-Omic Investigations

Yvonne J. Huang

Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan, USA

Correspondence: Yvonne J. Huang (yvjhuang@umich.edu)

Received: 11 September 2024 | **Revised:** 13 February 2025 | **Accepted:** 28 February 2025

Funding: This work was supported by the National Center for Advancing Translational Sciences, U01TR004066 and the National Heart, Lung, and Blood Institute, 75N92024D00012-0-759202400001-1.

Keywords: airways | lung | metabolomic | metagenomic | microbiota | transcriptomic

ABSTRACT

Asthma is one of the most prevalent and extensively studied chronic respiratory conditions, yet the heterogeneity of asthma remains biologically puzzling. Established factors like exogenous exposures and treatment adherence contribute to variability in asthma risk and clinical outcomes. It is also clear that the endogenous factors of genetics and immune system response patterns play key roles in asthma. Despite significant existing knowledge in the above, divergent clinical trajectories and outcomes are still observed, even among individuals with similar risk profiles, biomarkers, and optimal medical management. This suggests uncaptured biological interactions that contribute to asthma's heterogeneity, for which the role of host microbiota has lately attracted much research attention. This review will highlight recent evidence in this area, focusing on bedside-to-bench investigations that have leveraged omic technologies to uncover microbiome links to asthma outcomes and immunobiology. Studies centered on the respiratory system and the use of multi-omics are noted in particular. These represent a new generation of reverse-translational investigations revealing potential functional crosstalk in host microbiomes that may drive phenotypic heterogeneity in chronic diseases like asthma. Multi-omic data offer a wide lens into ecosystem interactions within a host. This informs new hypotheses and experimental work to elucidate mechanistic pathways for unresolved asthma endotypes. Further incorporation of multi-omics into patient-centered investigations can yield new insights that hopefully lead to even more precise, microbiome-informed strategies to reduce asthma burden.

1 | Introduction

Although asthma is one of the most common respiratory conditions worldwide, the risk of developing asthma, its prevalence, and disease burden vary greatly. The clinical heterogeneity of asthma is striking and is reflected in the different phenotypes that have been described, particularly in adults but also increasingly in children [1–3]. Such observations indicate that the etiopathogenesis of asthma is multifactorial and that different mechanisms, distinct or intersecting, shape asthma pathobiology and outcomes in established disease.

Clinical investigations to understand factors that shape asthma's heterogeneity have historically focused on either of the following: (1) early life exposures associated with risk for developing childhood-onset asthma, or (2) variables associated with different phenotypes, and potentially endotypes, of chronic asthma in adults. Earlier epidemiologic studies firmly established that early life microbial exposures, such as viral respiratory infections and microbial burden in the home environment, influence susceptibility to childhood asthma and related atopic diseases [4, 5]. This evidence has expanded to a large body of literature demonstrating the important role of host-associated microbiota,

particularly of the gut, in shaping early life immune responses and inflammatory pathways implicated in asthma [6, 7]. In children, atopic asthma related to type 2 (T2) immune responses is the most well-recognized endophenotype. However, it is now clear other asthma phenotypes exist wherein type 2 immune responses are much less prominent [8]. In contrast to T2-"high" disease, non-T2-"high" (also referred to as T2-"low") asthma is distinguished clinically by reduced levels of T2 biomarkers, such as blood eosinophils or fractional exhaled nitric oxide (FeNO) [1, 9, 10]. T2-low asthma is an umbrella for multiple phenotypes in which patients display suboptimal or poor clinical responses to standard therapies, including corticosteroids and biologics that target T2 pathways [11]. In this context, growing evidence has linked characteristics of host microbiota to asthma phenotype, in particular T2-low disease.

In general, knowledge of the gut microbiome far surpasses that of the respiratory microbiome, but both have been implicated in asthma pathogenesis and phenotype. A plethora of clinical and mechanistic studies have examined how gut microbiota can influence asthma risk and pathogenesis in early life [12–15]. In contrast, far less is understood about how gut microbiota might shape specific phenotypes of asthma in established disease despite much discussion of the "gut-lung" axis [16–18]. Additionally, findings from recent studies of the respiratory microbiome have brought this ecosystem into investigative focus as another potential modulator of asthma risk, phenotype and outcomes [19–26]. Clinical findings are buoyed when coupled with supporting data from experimental work, though the latter can be imperfect when modeling heterogeneous diseases. Conversely, microbial factors that experimentally mediate effects on immune responses often have yet to be confirmed in wider relevant clinical contexts. While bridging cross-translational gaps can be a challenge, the increasing accessibility of omic tools that can profile biological readouts at greater and deeper scales has led to their adoption across a variety of investigations. Hence, for complex chronic diseases like asthma, there is immense opportunity to leverage not just single-omic but multi-omic strategies to further understand the host biome. Integrative multi-omics, particularly when inclusive of functional readouts of a microbial community [27], can uncover pathways and mediators of host-microbial crosstalk (Figure 1). Deploying multi-omics in clinical studies could identify candidate pathways that differentiate disease endotypes for further targeted study in pre-clinical models.

This review will discuss first key evidence from clinical studies that have established associative connections between host microbiome features and asthma risk, outcomes, and phenotype. Evidence for implicated functional pathways will also be discussed where available. As the role of the gut microbiome in immune development and atopic disease risk has been extensively discussed elsewhere [6, 7, 18], a large portion of this review is dedicated to discussing the respiratory microbiome and recent omics studies that have provided evidence for its role in also shaping asthma in both early and later life. A perspective is offered on how the recent advances in knowledge and omics science should continue to motivate next-generation translational studies of asthma so that even more precise strategies can be developed to reduce disease risk and burden.

2 | Sampling Approaches to Study the Airways and Lungs in Asthma

To interpret the current literature, it is prudent to review what types of biospecimens have been used to characterize the host microbiome in studies of asthma. Knowledge of the human gut microbiome has largely come from fecal material and rarely from endoscopic sampling, which is invasive. Since the large intestine harbors the greatest collection of microbiota, fecal analysis is rational, convenient, and has been greatly informative in understanding interactions between gut microbiota and the

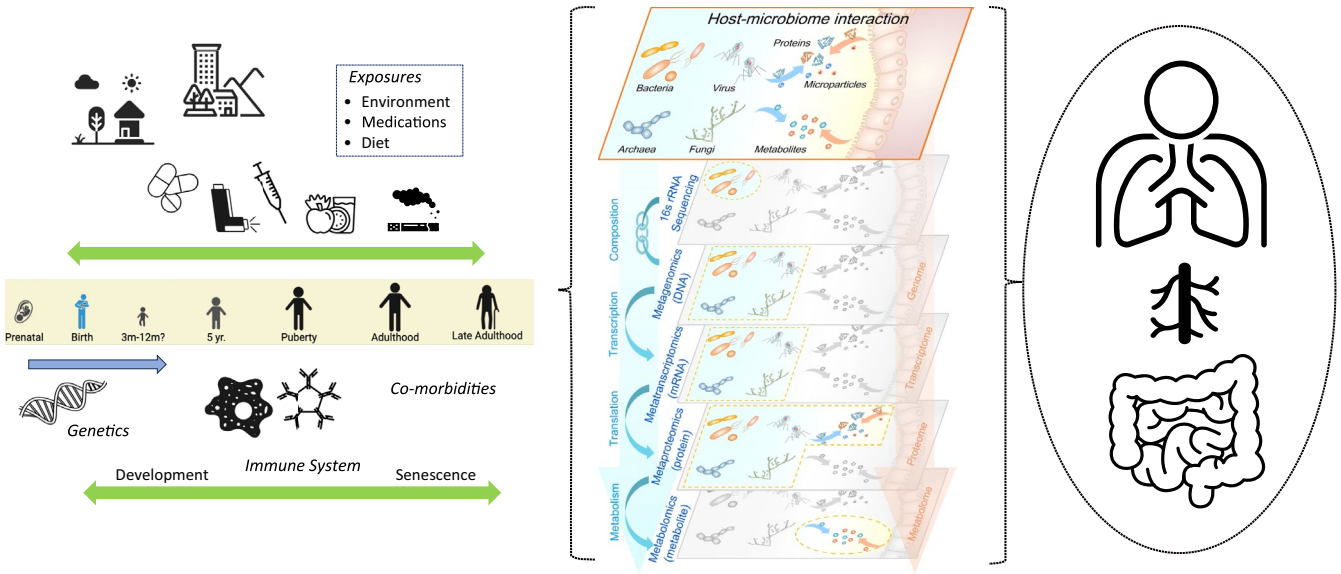

FIGURE 1 | Overview of the environmental and biological variables that broadly shape asthma risk and trajectory across the lifespan, and the role for multi-omic tools to understand the effects of functional host-microbiome crosstalk on asthma pathobiology and outcomes. Portion of the figure (graphic on "host-microbiome interaction") is borrowed from Zhang et al. 2019 [27]; under Creative Commons Attribution 4.0 International License which allows for unrestricted use.

immune system. However, fecal content does not directly reflect local interactions between, for example, intestinal epithelial cells and microbiota [28], which may be best studied with direct sampling that is more feasible in animal models.

Analogously, the anatomic complexity of the respiratory tract has guided the use of less invasive sampling methods. A variety of specimen types have served as the basis for characterization of the "airway" or "lung" microbiome reported across studies. These include nasal/nasopharyngeal swabs, nasal lavage, oral wash, or collection of lower airway samples via sputum induction or directly by bronchoscopy. It should be noted that described microbial compositions differ between samples of the upper and lower respiratory tract [29, 30]. Favored types of airway samples also differ between pediatric and adult studies, and the choice hinges on both clinical practicality and investigative focus. Accessing the upper respiratory tract is more tenable in infants and children and hence, nasal-derived samples have been the predominant biospecimen studied in large cohort studies [22, 31–33]. In adults, more invasive approaches can be tolerated, and thus studies analyzing sputum or bronchoscopy-obtained samples are more common [34]. Using more invasive approaches, however, limits the number of participant volunteers and the feasibility of longitudinal sample collections. Bronchoscopy allows for sampling by several approaches, resulting in different informative specimen types (bronchial epithelial brushings, endobronchial biopsies, or bronchoalveolar lavage fluid). In addition to sputum, these types of specimens capture different anatomic geographies, the types and numbers of immune cells represented, as well as differences in microbiota compositions [29, 30]. While microbiota compositions vary the most between individuals (regardless of the organ system including the respiratory tract), it is possible that niche-specific microbial functional redundancy is less variable. This has been described from the gut [35, 36] but not in the airways. Nonetheless, given the differences in immune cell types represented in the types of airway specimens studied in asthma, immune cell interactions with airway microbiota may differ between compartments of the respiratory system. This is likely to be reflected in omic profiles from different sample types.

3 | Role of the Microbiome in Early Life Asthma

3.1 | The Environment

It is well established that certain environmental factors and exposures are strongly associated with the risk for developing childhood asthma [4, 11]. Extensive evidence has also highlighted the development of the host microbiome, particularly the gut, over the first one to two years of life and its role in shaping the immune system during this critical time window [12, 13, 15, 18]. Relevant environmental influences or direct exposures can be categorized broadly as follows: (1) external environment characteristics that impact the opportunity for microbial contact (e.g., farm vs. nonfarm settings); (2) lifestyle practices that shape the establishment and maturation of the host microbiome (e.g., breastfeeding, pet ownership); (3) medical events or interventions that may affect the ecological succession of the developing microbiome and its interactions with the immune system (e.g., birth by cesarean section, early life respiratory virus infections, antibiotics). The above body of evidence underscores the multifactorial nature of early-life interactions that shape the risk for childhood asthma. This section will highlight specific studies that have pursued sophisticated strategies to interrogate the microbiome and microbiome–immune interactions, coupled in some cases with experimental work to understand mechanisms. They exemplify the power of combining interdisciplinary tools and expertise to gain insights into the complex host–microbiota interactions that underlie asthma susceptibility. These set a foundation for future studies to further understand such interactions in established disease and phenotypic heterogeneity.

The impact of environmental factors and exposures is clear from epidemiologic studies, in particular those comparing similar groups of individuals with different exposure opportunities or lifestyles. Investigations that included analysis of household dust content have provided further evidence of microbial links to the observed differences in asthma or atopy prevalence. For example, analysis of the microbial content of bedroom dust samples collected in two Western European cohorts found that children who lived on farms and who were less likely to have asthma or atopy were exposed to a greater variety of environmental microorganisms [5]. Notably, the diversity of microbes found in these samples (i.e., range of different types of both bacteria and fungi) was inversely related to the likelihood of having asthma.

Similar observations have been reported from other geographic regions including different populations with similar genetic ancestry [37–39]. For example, despite geographic proximity the Finnish and Russian Karelia populations exhibit different prevalences of atopy and asthma that has been attributed differences in living conditions and thus exposures (more westernized among Finnish Karelia) [37, 39]. The farm environment can be carried indoors, and a recent study aimed to quantify the "farm home microbiota" effect using microbial composition data measured from farm-home floor dust collected in a rural Finnish birth cohort [40]. The index ("FaRMI") derived from the measured relative abundances of bacteria/archaea was then applied to samples from a suburban (non-farm) Finnish birth cohort. Compared to suburban homes, rural farm-home dust was characterized by higher bacterial richness and enriched in members of specific bacterial groups (higher FaRMI). In the suburban cohort, higher FaRMI dust samples was negatively associated with asthma. Similar inverse associations with asthma were observed when FaRMI was derived from and applied to analysis of samples from an independent German birth cohort with farm and non-farm homes. The apparent asthma-protective effect of high FaRMI household dust was independent of atopic sensitization, suggestive of microbiota-specific contributions.

In the United States, studies of Amish and Hutterite communities have further bolstered the evidence for environmental/household influences on asthma risk or prevalence. Despite similar genetic ancestry, rates of allergy and asthma differ between Amish and Hutterite populations who practice traditional versus modern farming techniques, respectively [38, 41]. Although further research overall is still needed to understand what particular microbial components or products from an environment mediate or modulate asthma-protective mechanisms, studies from U.S. and European cohorts have elucidated some of the effects on host immune tone and responses in early life [41]. These

include baseline differences in expression of pattern recognition receptors measured in cord blood [42], and increased numbers and improved function of regulatory T cells in neonates born to farming mothers compared to those born to non-farm mothers [43]. Significant differences in the proportions and functional phenotype of peripheral blood innate immune cells were observed between Amish and Hutterite children [44]. As further proof-of-concept, using an ovalbumin mouse model of allergic asthma, intranasal instillation of house dust from Amish but not Hutterite homes significantly inhibited airway hyperreactivity and eosinophilia, effects not observed in mice deficient in MyD88 and Trif [44].

Further observations from U.S. pediatric cohorts focused on the urban inner-city environment have revealed additional complex associations, such as an interaction between environmental allergens and microbiota and the relationship to asthma risk. While high allergen environments are generally presumed to correlate with atopy and allergic asthma, a study from the Urban Environment and Childhood Asthma study found that house dust bacterial content interacted with aeroallergen sensitization to modify associations with atopy and asthma in young children [45]. Specifically, having high levels of both aeroallergen sensitization and bacterial richness in house dust (richness being a measure of how many different species are present in a sample) was negatively associated with recurrent wheeze, a clinical phenotype in young children suggestive of asthma. This study additionally identified candidate bacterial groups in the dust that may play a protective role; this included members of the bacterial families Prevotellaceae, Lachnospiraceae, and Ruminococcaceae [45]. Thus, at least in the urban environment, the opportunity for exposure to a wider range of bacteria may in theory modify the risk for allergy-related wheezing linked to pediatric asthma, but the mechanisms involved are unclear.

3.2 | The Gut Microbiome

The gut microbiome has been the most extensively studied of all human microbiomes, and its influence on early life immune development shapes the risk for atopic diseases including asthma. Of note, there is a scarcity of high-quality clinical studies examining the gut microbiome in chronic asthma, whether in children or adults. Several important considerations are warranted when interpreting gut microbiome data acquired beyond early childhood. As discussed later, a number of factors, cumulative with age, are independently linked to gut microbiome variation and thus increase the complexity of interpreting disease-specific associations.

Several pivotal studies, many from large birth cohorts, have helped to establish the gut microbiota's influence on early-life asthma risk, as summarized in brief here. One example is work from numerous studies conducted as part of the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) mother–child cohorts [31]. For example, from the $COPSAC_{2010}$ cohort, the investigators analyzed fecal samples from 690 infants collected at ages one week, one month, and one year [15]. The 1-year samples differed greatly from the earlier time points in gut bacterial composition, but two clusters were identified that captured this difference. Interestingly, this distinction was most apparent based on infants born to an asthmatic mother. In this group of at-risk infants, associations with asthma diagnosed at age 5 were characterized by differences in their 1-year relative abundances of specific gut bacterial groups like *Veillonella, Lachnospiraceae, Bifidobacterium*, and *Ruminococcus*. In contrast, infants in the group born to non-asthmatic mothers showed no significant links between their 1-year gut microbiota composition and a later asthma. Thus, this observed interaction between gut microbial community composition and a maternal history of asthma highlights intersecting factors between a potential predisposition to allergic disease and gut microbiota composition in early life.

Evidence from other birth cohorts further suggests the timeframe of a "critical window" for gut microbiome–host interactions that shape risk trajectory for early-onset asthma is much earlier, within the first 3 months of life. In a study of 319 infants enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) Study, there was a decreased prevalence of specific bacterial genera (*Lachnospira, Veillonella, Faecalibacterium, Rothia*) in fecal samples from age 3 months was associated with increased risk for asthma [12]. This was associated with reduced fecal levels of the short chain fatty acid (SCFA) acetate. In further experimental work, adult germ-free mice (dams) were inoculated with stool from an infant with atopic wheeze who displayed very low abundances of these 4 bacterial genera. The inoculation was conducted with or without supplementation with representative species from these 4 bacterial genera. Pups born to dams who received the bacteria-supplemented stool displayed reduced lung inflammation, higher levels of SCFAs, and reduced levels of proinflammatory lung cytokines. This type of work, coupling clinical observations with experimental work in preclinical models to support causal effects, augments the translatability of similar findings from animal models alone [46, 47].

Lastly, gut fungi (mycobiota) also play an important role in tuning the immune system [48], and fungal dysbiosis has been shown to aggravate allergic inflammation in experimental models [49]. However, human mycobiota are far less studied, and the role of specific gut fungi implicated in allergic airway disease varies across studies. Nonetheless, gut enrichment for *Candida* spp. has been associated with an increased risk for atopy in early life [13] and in mice, with increased numbers of group 2 innate lymphoid cells (ILC2) [50]. However, fungi and bacteria coexist, with structured colonization studies in mice showing that fungal colonization can promote significant shifts in gut bacterial community structure, overall microbiome assembly, and immune development [51]. In an analysis of both bacterial and fungal composition in stool samples collected in a U.S. birth cohort, a distinct gut microbiome and accompanying gut metabolomic profile were identified from early-life samples (within the first 3 months) that were associated with a higher risk of atopy in early life [13]. This dysbiotic profile was represented by lower relative abundance of specific bacteria (e.g., *Bifidobacterium, Akkermansia*, and *Faecalibacterium*), higher relative abundance of particular fungi (Candida and Rhodotorula), and a fecal metabolome enriched for pro-inflammatory metabolites. Interestingly, ex vivo culture of human blood T-cells with sterile fecal water from samples in this dysbiotic group led to an increased proportion of CD4+ cells producing interleukin–4 and a reduced percentage of Foxp3+, CD25+, and CD4+ cells. The

fecal metabolite [12, 13] DiHOME was identified as a potential mediator of the effect on regulatory T-cell abundance.

3.3 | The Upper Airway Microbiome

More recently, the respiratory microbiome in infants and children has been examined to understand its potential role in shaping asthma development and outcomes. As mentioned earlier, studies of the respiratory microbiome in children overwhelmingly sample the upper airways (from nasal passages to the supraglottic hypopharyngeal region). Microbiota composition is distinct in these niches when compared to the lower airways, at least in adults [29, 30]. It is known that nasal epithelial gene expression can recapitulate findings from the analysis of bronchial epithelial cells [52] and serve as a reasonable surrogate of immune responses in asthma. However, to what extent differences in upper and lower airway microbiota composition might trigger compartment-specific differences in epithelial or local tissue immune responses is not known. Speculatively, potential functional redundancy in the airway microbiome might result in modest differences in host responses between the upper and lower airways, but this remains unstudied. On the other hand, such differences may be magnified in severe asthma, where more significant changes have been described in the lower airways, both microbially and immunologically [20, 26, 53]. Further understanding of potential topographical variation in host-microbiome functional interactions would be useful as they could be associated with different phenotypic aspects of asthma (e.g., atopic vs. non-atopic asthma, type 2- high vs. type 2-low inflammatory phenotypes, asthma associated with obesity or metabolic dysfunction).

Despite the limitations of upper airway sampling (e.g., nasal swabs or spontaneous nasal secretions from nasal "blow"), studies from large pediatric cohorts have consistently demonstrated relationships between nasal bacterial composition and outcomes in pediatric asthma. This includes the influence of respiratory viral infections, an important risk factor, on the nasal microbiota. The influence of early life respiratory viral infections and immune response patterns on asthma risk has been discussed extensively in the literature [4, 54].

The composition of nasopharyngeal (NP) bacteria, like in the gut, is dynamic in early life and similarly influenced by external and environmental factors. In a study of 112 healthy infants sampled frequently in the first year of life, factors differentially associated with NP bacterial composition were the mode of delivery, infant feeding, crowding, and recent antibiotic use [55]. In contrast to the lower airways, *Corynebacterium* and *Dolosigranulum* are more prevalent members of the NP microbiome and seem to be associated with healthy states as well as with better clinical outcomes. This has been described across several studies [30, 32, 56]. Children experiencing more respiratory tract infections (RTIs) in the first year of life also displayed an aberrant microbiota development trajectory very early on at age one month [55]. Risk for RTIs involved decreased temporal stability of the NP microbial community, sustained lower prevalence of *Corynebacterium* and *Dolosigranulum*, and early enrichment in *Moraxella*. Intriguingly, NP enrichment in *Corynebacterium* and *Dolosigranulum* also has been associated with reduced frequency of asthma exacerbations [32] or lower probability of loss of asthma control in pediatric patients [56]. These indications that NP enrichment in *Corynebacterium* may have a protective effect also extend to findings in adults. For example, in a study of adults with or without mild asthma, we observed the genus *Corynebacterium* dominated NP samples in all healthy individuals but in only 50% of adults with mild atopic asthma [30]. Further, an inverse relationship existed between the NP relative abundances of *Corynebacterium* and *Moraxella*, and these two genera also displayed contrasting directional relationships with levels of pro-inflammatory cytokines in paired bronchoalveolar lavage (BAL) fluid. For example, BAL eosinophil percentages correlated negatively with NP abundance of *Corynebacterium* and positively with NP abundance of *Moraxella*. *Corynebacterium* negatively correlated with BAL levels of IL-7, IL-21, and IL-6, while *Moraxella* was positively correlated with TNF- and CXCL11. Collectively, findings from the above studies indicate that upper airway microbiota correlate with asthma-related outcomes and also immune response patterns in the lower respiratory tract.

Other clinical studies have aimed to further dissect how the upper airway microbiota, including virus-bacteria interactions, might modulate pediatric asthma risk and outcomes. For example, in a multicenter cohort of infants hospitalized with respiratory syncytial virus (RSV)-induced bronchiolitis, having a *Haemophilus*-dominant NP microbiota at the time of admission was associated with delayed clearance of RSV [57]. This suggests that an individual's existing NP bacterial community may play a role in determining the outcome or duration of symptoms with RSV infection. An earlier study of rhinovirus (RV) infection in 308 children with and without asthma found that co-detection of specific bacterial pathogens in nasal samples, in particular *Moraxella catarrhalis* and *Streptococcus pneumoniae*, augmented cold and asthma symptoms compared to detection of only RV [58]. In an analysis of NP samples collected longitudinally from infants in the Australian Childhood Asthma Study [21], similar associations of *Streptococcus, Haemophilus*, or *Moraxella* with viral RTIs were found. However, the consequences of having an RTI-associated NP bacterial pattern dominated by these three bacterial groups differed by atopic status. Children with evidence of early atopic sensitization were more likely to have a "persistent wheeze" (pre-asthma) phenotype by age 5 years, in contrast to non-early-sensitized children with the same NP bacterial pattern. This bacterial modulation (statistically) of asthma risk, dependent on underlying atopic status, is similar to that also observed with the early life gut microbiota, as discussed earlier [15].

Much remains unknown mechanistically about how microbiota and bacterial-virus interactions influence asthma pathogenesis. In human studies, incorporating readouts of immune responses and molecular mediators with microbial data is increasingly pursued. Such data range from targeted assay approaches to integration of omic data types. Example studies include the following. From the COPSAC cohort, analysis of hypopharyngeal (oral) aspirates found that higher relative abundances of *Veillonella* and *Prevotella* in one-month-old samples were associated with asthma diagnosis at age 6 years and with reduced TNF-α and IL-1β and increased CCL2 and CCL17 in nasal epithelial lining fluid [22]. Another study

of infant NP samples collected at age 1 week, 1 month, and 3 months found that reduced bacterial richness in 1-week-old samples (richness is an alpha-diversity measure reflecting number of different species in a sample) was associated with having allergic rhinitis at age 6 years, which was mediated by an epigenetic signature related to the expression of genes for lysosome and bacterial invasion of epithelial cell pathways [59]. Hypothesis-driven clinical studies have also explored the role of altered sphingolipid synthesis, implicated in asthma pathogenesis through genetically regulated activity of serine palmitoyl-CoA transferase (SPT) [60]. Lower concentrations of plasma sphingolipids at age 6 months were linked to increased risk of asthma in early childhood, the 17q21 genotype that is strongly associated with asthma, and reduced expression in nasal epithelial cells of SPT, the rate-limiting enzyme for de novo sphingolipid synthesis. While the microbiome was not examined in the particular study by Rago et al., it should be noted that sphingolipids can be produced or utilized by certain bacteria [61, 62] and thus hypothetically could impact systemic concentrations of sphingolipids.

Other studies have aimed to integrate upper airway microbiota information with metabolomic or transcriptomic data to further glean potential molecular interactions or endotypes related to asthma outcomes. For example, as viral bronchiolitis in infants is an important risk factor for asthma, two recent studies used integrated-omics approaches to examine illness endotypes related to RV or RSV infection. In a study that integrated data on NP bacterial composition (16S ribosomal RNA gene sequencing), nasal cytokines, and untargeted metabolomics, Raita et al. [63] found that an RV bronchiolitis endotype characterized by rhinovirus-C infection, a *Moraxella*-dominant NP microbiota, higher levels of T2 and epithelial-derived cytokines, and lipid metabolites was associated with a higher risk for recurrent wheeze and asthma. Another study used dual RNA-seq (also referred to meta-transcriptomics) to examine NP microbial and host gene expression in relation to the severity of bronchiolitis [64]. This approach provides at least three types of data: taxonomic profiles of microbial composition, as well as functional readouts of expressed microbial and host genes. This study observed that host and microbial functional modules associated with the risk for clinically severe bronchiolitis included, on the host side, decreased type I interferon and regulatory T-cell responses, and increased neutrophil/IL-1 responses. On the microbial side, increased abundance of *S. pneumoniae* and evidence of microbial branched-chain amino acid metabolism were some of the factors associated with more severe bronchiolitis. Finally, in a study of children with exacerbation-prone asthma, nasal microbiota composition varied by season and in relation to viral-associated asthma exacerbations [33]. Analysis for microbiota associated with host gene expression modules identified a bacterial network that included *Streptococcus, Haemophilus*, and *Neisseria* members that were linked to an exacerbation-associated SMAD3 expression module.

In sum, the above-discussed studies on the early-life microbiome offer examples of how discovery-based approaches have uncovered key microbial themes, many consistent across different study cohorts, that are linked to the risk of developing childhood asthma. Some studies were able to incorporate experimental work to support the biological plausibility of observed associations between specific microbiota, host responses, and metabolite mediators. Others leveraged multi-omic tools coupled with sophisticated computational approaches to dissect host-microbial molecular interactions related to asthma outcomes. Even in early life, it is evident that host-microbiome interactions are complex, bidirectional, non-linear, and depend also on external variables that differ between individuals. These multifaceted interactions do not easily lend themselves to reductionist approaches to dissect all potential mechanisms. Nonetheless, these early-life studies serve as a reference for investigative strategies to understand the even greater complexities of asthma heterogeneity in later life, where additional factors shape differences in disease phenotype. Table 1 highlights some of the notable pediatric studies discussed above, as well as those in adult asthma, discussed next, that offer examples of useful investigative strategies going forward.

4 | The Microbiome and Chronic Asthma in Adults

4.1 | Overview

Compared to the very large number of studies focused on childhood asthma, fewer have investigated how the microbiome might further shape asthma in patients with established disease. This includes differences in asthma phenotypes and related immunobiology, as has been described from various adult cohorts [2, 65, 67–70]. As mentioned earlier, sample collections in adult studies have largely targeted the lower airways, using either bronchoscopy or sputum induction. Compositionally, microbiota differ greatly between the upper and lower airways but are more similar across lower airway specimen types. The evidence establishing this comes from several carefully conducted studies in both healthy adults and persons with chronic asthma [19, 29, 30, 71]. They included the use of bronchoscopy to directly sample the lower airways, in the process collecting technical controls to enable clearer interpretation of lung-derived signals [34]. The lung is continuously exposed to inhaled air and oropharyngeal secretions but harbors much lower microbial biomass than the colon. Thus, attention to procedural aspects of specimen collection and processing is important, such as inclusion of technical controls to understand background microbial DNA signals (e.g., from instruments and reagents used during collection and processing).

A gradient of bacterial burden (abundance) exists along the tracheobronchial tree into the lungs, and the compositional structure of microbiota differs across specimen types (e.g., sputum versus bronchoalveolar lavage fluid) [29, 30]. This likely reflects to a large degree differences in sampling area and volume and their reflection of lung topography. Nonetheless, in a study [30] that thoughtfully compared bacterial microbiota identified from paired (within-patient) bronchial epithelial brushings, sputum, oral wash, and nasal brushings, it was demonstrated that in persons with asthma, sputum and bronchial brush microbiota were more similar than the same comparison of these specimen types in healthy subjects. Simply put, this showed that in the presence of chronic airway disease, even of mild severity, induced sputum can reasonably reflect bronchial mucosa-associated microbiota. The observation is important because

TABLE 1 | Notable clinical studies of microbiome-host interactions in asthma using multi-omics and data integration tools.

Topic	Methods	Reference
Gut microbiome and early-life asthma; 319 infants (CHILD study)	Gut microbiome (16S rRNA gene sequencing; in silico predicted metagenomics), short-chain fatty acids and urine metabolomics, human fecal microbiota experimental model of murine asthma	[12]
Gut microbiome and risk for childhood allergy/asthma; 308 infants (WHEALS cohort)	Gut microbiome (16S rRNA and fungal internal transcribed spacer region (ITS) sequencing; in silico predicted metagenomics), stool metabolomics, ex vivo dendritic cell and T-cell co-culture experiments	[13]
Nasal microbiota and risk for childhood asthma; 544 children (COPSAC2010 cohort)	Nasal bacteria (16S rRNA gene sequencing), nasal cytokines (mutiplex assays)	[22]
Nasal microbiota-transcriptome and asthma exacerbations;181 children (ICAC consortium)	Nasal microbiota (16S rRNA and ITS sequencing), nasal transcriptome modules	[33]
Nasal microbiota-epigenome and allergic disease risk; 500+ children (COPSAC2010 cohort)	Nasal bacteria (16S rRNA gene sequencing), nasal epigenome modules	[59]
Nasal microbiome-metabolome and viral bronchiolitis; 121 children (MARC-35 consortium)	Nasal bacteria (16S rRNA gene sequencing), nasal cytokines and metabolomics	[63]
Nasal meta-transcriptome and bronchiolitis severity; 244 children (MARC-35 consortium)	Nasal meta-transcriptomics (microbial composition, microbial and host gene expression)	[64]
Bronchial microbiota and epithelial transcriptome in severe asthma; 30 adults	Bronchial brush bacteria (16S rRNA gene sequencing), epithelial transcriptome (miroarray)	[20]
Bronchial or sputum microbiome, type 2 and non-type 2 inflammation and corticosteroid effects in mild asthma; 84 adults (NHLBI AsthmaNet clinical trial)	Bronchial brush or sputum bacteria (16S rRNA gene sequencing), airway gene expression, ICS intervention	[24, 25]
Sputum microbiome-transcriptome-proteome and asthma severity/phenotypes; 72 adults (U-BIOPRED)	Sputum microbiome (16S rRNA and metagenomic sequencing), transcriptome (microarray), proteome	[65]
Sputum microbiome and cytokine responses in obese asthma; 61 adults	Sputum microbiome (16S rRNA and ITS sequencing), sputum and blood cytokines, bacterial-cytokine network analysis	[66]

sputum is the most commonly relied upon specimen in studies of human airway disease biology, including asthma, for its less invasive collection and therefore ability to obtain in larger numbers of patients. Thus, for heterogeneous diseases like asthma, sputum microbiome data has been analyzed together with other molecular readouts to gain insights into whether and how lower airway microbiota potentially shape airway disease phenotype [25, 26, 66, 72–74]. Furthermore, this can be pursued in larger numbers of patients than could be logistically pursued by bronchoscopy.

It is important to note that many prior studies of the lower airway microbiome in adults with chronic lung disease, including asthma, have generally involved patients with more advanced disease (e.g., moderate to severe asthma or chronic obstructive pulmonary disease). Thus, reported "disease-associated" microbiome differences should always be interpreted with knowledge of cohort characteristics like disease severity and related factors like medical therapies or comorbidities. With this overview, some of the main studies that have contributed to the current understanding of the microbiome's role in chronic asthma are reviewed next.

4.2 | The Lower Airway Microbiome in Chronic Asthma and Adult Phenotypes

One of the first multicenter studies that took advantage of bronchoscopy-collected epithelial brushings to explore the bronchial microbiome examined this in 65 adults (42 with suboptimally controlled asthma) [19]. Using a microarray approach to characterize bronchial bacterial content, this study found the following: (1) bacterial burden was higher in asthmatic than in healthy control participants; (2) differences in bronchial bacterial composition and higher bacterial diversity (based on the Shannon index) correlated with greater airway hyperresponsiveness measured by methacholine bronchoprovocation; and (3) in this secondary analysis of brushes obtained as part of a

parent clinical trial of clarithromycin therapy for sub-optimally controlled asthma [75], macrolide-related improvement in airway hyperreactivity was linked to possession of a greater diversity of bacteria at baseline [19]. This investigation was among the first to suggest and support the hypothesis that airway microbiota composition may correlate with clinical outcomes in adults with chronic asthma.

A subsequent study also analyzed bronchial brushings collected in another multicenter study focused on severe asthma to explore whether mucosa-associated microbiota correlated with phenotypic features in severe asthma [20]. Although the sample size of 30 patients was modest, excellent phenotyping data (clinical and immunologic) enabled more detailed analysis for relationships to the bacterial microbiome, which at the time had yet to be merged investigatively in adult asthma. Several intriguing observations emerged from this study that informed subsequent investigations pursued in the field. First-pass high-level analyses identified several clinical and immunologic variables associated with variation in the overall compositional structure of bronchial bacteria (also referred to as microbial community structure). Host variables associated with this bacterial community variation includednumbers of sputum neutrophils, numbers of eosinophils in endobronchial biopsies, bronchial epithelial gene expression signatures, and clinically, asthma symptom control and body mass index. With respect to markers of inflammatory or immune phenotype, bronchial enrichment in members of the Proteobacteria phylum strongly correlated with poor asthma control and an epithelial expression signature for Th17-related genes. In contrast, evidence of epithelial response to corticosteroids (*FKBP5* gene expression) correlated with bronchial enrichment in members of the Actinobacteria phylum. Phylogenetically, Proteobacteria and Actinobacteria represent divergent lineages with attendant differences in functional capacities of their member species. Interestingly, bacterial correlations with a third gene expression signature for type 2 responses were not found in this study, and this was congruent with the inverse relationship separately observed between numbers of airway eosinophils (quantified from biopsies or sputum) and bacterial burden. This suggested that eosinophilic airway inflammation, in this severe asthma cohort, was associated with reduced bronchial bacterial burden. Lastly, this study also observed that severe asthma participants who were obese, compared to their non-obese counterparts, displayed bronchial enrichment in members of the Bacteroidetes and Firmicutes phyla (in contrast to the other two phyla discussed above). This finding provided a first indication that obesity, an important comorbidity, might itself be associated with altered bronchial microbiome characteristics, suggesting the latter could be involved in pathways for obesity-complicated asthma.

Data from another cohort has since provided further validation that the lower airway microbiome is perturbed in moderate to severe asthma [26, 53, 76, 77]. This finding is broadly consistent across different cohorts, analyses of different lower airway sample types, and various computational approaches. Moreover, altered lung microbiota composition in more severe disease has similarly been observed in other conditions like COPD [78–81]. Lingering questions from these earlier studies included to what extent changes in the airway microbiome reflect consequences of restricted airflow, other disease-related biology, or even prescribed therapies, all of which might act as selective pressures on the microbiome in sicker patients. These questions informed the design and completion of subsequent investigations in adults with asthma, including several conducted by our teams.

The issue of disease severity and chronic treatment effects was deemed an important priority. The related questions are: is the lower airway microbiome different (altered) even in clinically mild airway disease when compared to unaffected controls? Do inhaled therapies change the airway microbiome, in particular corticosteroids, which are ubiquitously prescribed and immunomodulatory? Disentangling this in cross-sectional studies is challenging, if not impossible, but longitudinal or interventional study designs are advantageous. The latter is also useful because of the great variability between persons in their microbiomes, for which repeat sampling is analytically useful to control for this. These considerations informed a study conducted by the National Heart, Lung, and Blood Institute AsthmaNet network [24], that enrolled adults with or without mild asthma from across nine U.S. academic institutions. Detailed clinical assessments and biospecimen collections were performed, including the use of bronchoscopy and sputum induction. A total of 84 adults were enrolled: 42 with steroid-naïve, mild atopic asthma, and the remainder non-asthmatic subjects who were either atopic or non-atopic based on serologic evidence of aeroallergen sensitization. It was reasoned that the inclusion of atopic controls without asthma was an important comparator group, given the common presence of atopy in asthma but conversely, not all atopic individuals develop asthma. Following a baseline visit and sample collections, the mild asthma group (previously naïve to inhaled corticosteroid use) was then randomized to either inhaled fluticasone propionate or placebo treatment for six weeks, after which repeat clinical assessment and sample collections were pursued. In addition to microbiome analysis, baseline levels of type 2 airway inflammation were determined based on bronchial expression of three IL-13 responsive epithelial genes, as previously described [82, 83]. Physiologic evidence of response to fluticasone was assessed by repeat methacholine bronchoprovocation to determine whether airway reactivity was improved.

The first set of findings from this study focused on data from the bronchial epithelial brushings [24]. Using a phylogeny-based measure of bacterial diversity, there was evidence that even in mild asthma, the types of bacteria present differed from non-asthmatic controls. More granular analyses (i.e., genus or taxon/species-level analyses) further identified specific microbiota members that were differentially prevalent between the asthmatic and atopic/non-asthmatic groups. Another interesting observation was the finding that differences in bronchial bacterial composition also were found between the atopic and non-atopic groups who did not have asthma. This finding suggests that atopy itself may be linked to an altered composition of airway mucosal microbiota, with further perturbations occurring in asthma.

Phenotyping asthma by level of type 2 (T2) inflammation is clinically informative because identifying patients with a T2-high endophenotype benefits from tailored treatments targeting T2 inflammation. Hence, participants in this study [24] were also categorized as T2-high or T2-low, based on expression levels

of type-2 inflammation-related genes [83]. Most of these adult subjects actually had evidence of low T2 airway inflammation, which aligns with literature that T2-low asthma may predominate in many adults while others display more consistent T2 biomarkers [8, 69]. Classifying the asthmatic group by T2 status led to a third notable finding that bronchial bacterial burden at baseline was significantly higher in those with a T2-low profile. Conversely, bacterial burden was lower in those with a T2-high profile, harkening back to the earlier discussed findings in severe asthma of an inverse relationship between airway eosinophilia and bacterial burden [20]. These concordant findings strongly suggest that bacterial members of the airway microbiome could play a greater role in T2-low asthma phenotypes, even in clinically mild disease.

Lastly, Durack et al. examined the effect of the inhaled corticosteroid fluticasone on the composition of microbiota recovered from bronchial epithelial brushings and sputum [24, 25]. Changes in airway bacterial community structure and the relative abundance of specific microbiota members were observed with fluticasone treatment. This was distinct from any compositional shifts observed in the placebo-treated group, indicating that even short-term inhaled steroid therapy might induce changes in the microbiome. Perhaps even more interesting was the observed association between baseline microbiome characteristics and response to the ICS. Specifically, those who were ICS non-responders possessed a significantly different airway microbial community at baseline (pre-treatment sample) compared to ICS responders. The non-responders also demonstrated a greater change in their airway microbial community structure after fluticasone treatment, indicative of more significant shift in airway bacterial composition. Using an in silico approach to infer predicted bacterial metagenomes, putative microbial functions represented in the airway microbiome were examined to discover potential microbial pathways associated with non-response to ICS. This revealed predicted bacterial functions (KEGG pathways) involved in drug metabolism and degradation of xenobiotic compounds [24]. Together, these findings suggested that lower airway microbiota may harbor functional capacities that influence treatment responses, potentially contributing to the heterogeneity observed clinically in asthma.

Understanding potential mechanisms through which microbiota modulate host responses, and by extension disease phenotypes or outcomes, is inherently challenging due to the inherent complexities of any ecosystem where multiple pathways intersect, are dynamic, and potentially synergistic or antagonistic for the outcome in question. Despite such challenges, adapting observations from human studies into model systems for further study can help support mechanistic plausibility for the clinical association. Pursuant to the above observations, subsequent work has aimed to begin to understand the biological basis or consequences of noted interactions between corticosteroid exposure, the airway microbiome, and asthma-related outcomes via in vitro and in vivo studies [84–86].

Repository strains of bacteria are often used in experimental studies, but such strains may not exhibit properties or behaviors similar to primary human isolates of the same species. To test the overall hypothesis that corticosteroids may directly affect airway microbes and their behaviors, bacteria were culture-isolated from sputum or BAL samples collected from adults with non-severe asthma for subsequent in vitro work [84]. The study focused on gram-negative bacteria because these represent many potential respiratory pathogens that belong to the Proteobacteria phylum. As discussed earlier, bronchial enrichment in Proteobacteria correlated with worse asthma control and an expression signature for Th17-related epithelial genes in severe asthma [20], representing a steroid-resistant phenotype. Several gram-negative species were successfully cultured from asthma patient samples, including members of the following genera: *Klebsiella, Pseudomonas, Stenotrophomonas*, and *Acinetobacter*. To specifically examine the effect of fluticasone, minimal media for culture was supplemented with fluticasone as the sole primary carbon source. The majority of tested isolates exhibited growth changes when compared to vehicle conditions, the magnitude of which varied between species. Three ATCC strains of clinically relevant species (*Klebsiella pneumoniae, Pseudomonas aeruginosa, Stenotrophomonas maltophilia*) also tested; all demonstrated significant growth responses with fluticasone added. Because *K. pneumoniae* has been implicated in ICS-associated pneumonia in clinical trials of fluticasone-based therapies in COPD [87], more in-depth experiments were pursued using this strain as a model organism. A series of experiments revealed that fluticasone exposure resulted in altered expression of several bacterial genes by RNAseq analysis and reduced the metabolic activity of bronchial epithelial cells and their expression of human β-defensin-2. Interestingly, targeted LC–MS assays also detected a known fluticasone metabolite from *K. pneumoniae* cells exposed to fluticasone only, whereas this metabolite was not detected in other derivatives or uninoculated media containing fluticasone. Thus, this proof-of-concept, pilot study indicated that this particular strain of a disease-relevant pathogen possessed the functional capacity to respond to and metabolize fluticasone. These observations lend support to the broader concept that airway microbiota may represent an off-target effect of inhaled therapies, which may have potential implications for treatment efficacy in chronic airway diseases.

4.3 | Obesity, Asthma and the Microbiome

In addition to the inter-relationships observed between the lower airway microbiome, level of type 2 inflammation, and response to corticosteroids, another important phenotype with a plausible connection to the microbiome is obesity-related asthma (OA). Obesity is associated with an augmented risk for developing asthma and also complicates asthma management because responses to treatment can be poor [88]. Additional immune and metabolism-related pathways beyond type 2 inflammation have been implicated in OA [89–92]. As one example, systemic IL-6 inflammation and features of metabolic dysfunction have been observed in an IL-6-high subgroup of severe asthma patients with co-morbid obesity [93].

In our prior study of adults with severe asthma, a signal for obesity-associated differences in the bronchial microbiome was detected [20]. To follow up on this observation, a recent study examined if similar obesity-related differences in airway microbiota exist in milder asthma or in the absence of asthma [66]. Airway microbiota-immune mediator relationships also were examined to determine if potential microbial-immune crosstalk

differs between obese/non-obese individuals with or without asthma. This study found that obesity itself, irrespective of asthma status, was linked to significant differences in sputum bacterial community structure and composition. Obesity-related airway enrichment in several organisms included species that belong to the same bacterial phyla as those represented in the earlier finding in obese severe asthma [20]. However, within the asthma group, further airway microbiota differences were identified between obese and non-obese participants, not only in bacterial composition but also in fungal richness. Predicted microbial metagenomic functions enriched in obese asthma included several pathways for fatty acid synthesis and metabolism, lipoic acid and glutathione metabolism, and insulin-related signaling. These pathways invite speculation that airway microbiota might somehow play a role in modulating the airway milieu and inflammatory responses in OA patients. Cytokine-cytokine correlations differed between OA and non-obese asthma patients, but further integration of bacterial microbiota data revealed a greater number of bacterial-connected cytokine networks in OA. For example, within-cluster connections were observed between members of the *Prevotella* and *Rothia* genera and the cytokines PAI-1, IL-1β, TNF-α, GM-CSF, IL-6, and IL-8.

In closing this section's discussion on chronic asthma in adults, it is noted that discussion of the gut microbiome in adults has been omitted because few robust studies exist due to modest sample sizes and/or insufficient accounting for potential confounders. These issues are a limitation and pose a challenge for deciphering human gut microbiome relationships to chronic lung disease biology in later life. At the same time, controlling for all potential non-disease-related factors that can shape the human gut microbiome is nearly impossible in clinical investigations. The next section will focus on the increasing interest in adopting multi-omic approaches to understand asthma heterogeneity, including integration of microbiome data, which will enhance understanding of how host-microbiome interactions might shape asthma outcomes.

5 | The Next Frontier: Multi-Omics and Functional Understanding of Host-Microbiota Interactions in the Airway

The term meta-omics has been used to describe the collective use of high-throughput techniques to study the complexity of microbial communities, with a focus on microbial functional properties [27]. Moving beyond compositional characterization by targeted amplicon sequencing (e.g., 16S rRNA for bacterial, 18S rRNA, or ITS for fungi), methods like shotgun metagenomics, metatranscriptomics, metaproteomics, and metabolomics are gaining attention (Figure 1). Each of these provides different information about a microbial community and its functions, from genomics-based predicted functional capacities to detection of expressed products. Advantages and disadvantages exist for each method. One major challenge for airway studies is whether there is sufficient appropriate material from particular specimen types to pursue any or all of these omic tools. Moreover, considerable computational skills and statistical knowledge are needed for bioinformatic processing and optimal analysis of such high-dimensional data, particularly with multi-omic data integration. These issues are important and have been discussed in great detail elsewhere [27, 34, 94, 95]. As apparent from the evidence reviewed up to here, the vast majority of microbiome studies to date in asthma and other chronic diseases have generally reported findings from one approach, typically 16S rRNA gene sequencing, which is cost-effective, scalable, and relatively straightforward to analyze nowadays. Many other studies have used single -omic platforms to understand host responses through gene expression profiling and metabolomics, with now exciting single-cell and spatial methods for these now available. While the future of this field lies in merging data from functional readouts of both microbiota and host to better understand their crosstalk in disease or health, the above significant methodological challenges continue to be addressed. Nonetheless, an important first step is the conceptualization of the host as an ecosystem, a perspective that should inform future study designs whether in the clinical domain or in experimental work conducted in pre-clinical models.

Recent studies focused on asthma or other chronic airway diseases offer examples of multi-omic investigations pursued from an ecological perspective. Some of these pediatric studies were discussed earlier, such as the finding that an allergy-associated nasal epigenetic signature at age 6 was mediated by a reduced airway bacterial diversity present at age 1 week [59]. Other investigations were able to generate and analyze functional multi-omics data from the same specimens and timepoints. Notably, this was successfully conducted in studies of the nasal ecosystem during viral bronchiolitis (microbiome, transcriptome, metabolome) [63, 64] and has also been performed from stool samples (gut microbiome and metabolome) to understand atopy risk in childhood [13] or severity of COPD in adults [96].

Multi-omic studies of samples collected from the lower airways or lungs of patients are very much dependent on the amount of material retrieved, storage processing steps, and also overall sample size. Invasive sampling inevitably dictates smaller numbers of willing research volunteers and may potentially represent a more limited spectrum of disease or clinical state depending on investigative goals. Nonetheless, recent studies in adults offer important examples of how the field is pushing forward multi-omic study of lung-derived samples, including microbiome data. Findings from such studies have revealed new potential molecular markers or pathways associated with asthma heterogeneity. For example, from the Unbiased BIOmarkers in PREDiction of respiratory disease outcomes (U-BIOPRED) study of asthma, researchers identified a severe asthma-related sputum microbiota cluster defined by reduced bacterial diversity, enrichment in pathogenic bacteria (e.g., *Haemophilus influenzae, Moraxella catarrhalis*) sputum neutrophilia, and worse asthma outcomes [26]. Subsequent analysis of available paired-omics data in U-BIOPRED revealed sputum biomarkers related to this pathogen-enriched cluster, such as 11-dehydrothromboxane B2 and prostaglandin (PG) E2 and D2 from lipidomics for eicosanoids [72]. Paired microarray gene expression data revealed differentially upregulated pathways related to immune regulation and inflammation, for example, tumor necrosis factor (TNF)-α and related regulatory genes; interleukins (ILs) and related regulatory genes; Toll-like receptors (e.g., *TLR2*, *TLR4* and *TLR10*); and inflammasomes (e.g., *LRP3*, *NLRP12* and *NLRC5*). Paired sputum proteomic data found associated enrichment in proteins related to neutrophilia, inflammation, and Th17 and Th1

mediated pathways. In contrast, this pathogen-enriched sputum cluster displayed downregulated pathways for cell growth, proliferation, DNA repair, OXPHOS, tricarboxylic acid cycle, and ROS pathways, suggesting impaired functions for cellular repair and mitochondrial dysfunction. Of note, this pathogen-enriched cluster was small, comprised of only 25 severe asthma patients. In a recent subsequent study from U-BIOPRED, for which 5 types of omics data were analyzed together, the inherent challenges of maximizing available paired-omics data from the same sputum specimen were apparent [65]. Out of 267 participants with available induced sputum, single-omic data could be generated from the majority of samples (range 120–246) for transcriptomics (microarray), proteomics (shotgun and SomaScan), and microbiome (16S rRNA and metagenomics). However, only 85 participants (72 asthmatic) had available sputum multi-omic data from all of the above. Despite this limitation, using sophisticated analytical approaches, five stable omics-associated clusters were identified, three involving severe asthma patients. The earlier described pathogen-enriched cluster was re-identified (albeit even smaller in participant numbers), along with another neutrophilic severe asthma cluster associated with IL-22 activation.

Thus, interesting patterns of co-associated molecular interactions, with or without microbiome links, can be gleaned through advanced integrative analysis approaches, even if sample sizes are modest. Validation efforts using other cohort data would augment understanding of the broader significance of putative endophenotypes identified from multi-omic analyses, but the lack of available similar data from comparable independent cohorts is often a problem. Here, it is worth taking note of the growing number of studies in other airway diseases, such as bronchiectasis and COPD, that have also leveraged multi-omics, including functional microbiome data, to elucidate microbiota-host connections underlying their disease heterogeneity. For example, two recent studies of mild to moderate COPD patients analyzed bronchoscopy-collected samples for paired lung microbiota and metabolome [97] and paired bronchial microbiota and epithelial transcriptome analyses [98]. Both independently identified relationships between specific bacterial genera (e.g., *Prevotella*, *Streptococcus*) and metabolomic or gene expression features that together associated with COPD status or severity. Specifically in both cohorts, bronchial enrichment in members of the *Prevotella* genus was negatively associated with COPD diagnosis or its severity. In the U.S. cohort, enrichment in *Prevotella* was positively associated with quantitated levels of adenosine and adenosine-related metabolites in bronchoalveolar lavage fluid [97]. In the European cohort [98], only in participants receiving ICS therapy was the relative abundance of *Prevotella* found to be positively associated with the expression of epithelial genes involved in tight junction promotion. This relationship was not observed in COPD participants not on ICS therapy, which suggests the potential functional interaction is more pertinent in symptomatic COPD as an indication for ICS treatment.

Taking findings from bedside-to-bench is important when feasible, and increasingly this has been pursued to understand functional processes or consequences of clinically observed links between the airway microbiome and disease outcomes. For example, based on our findings that ICS treatment can change the bronchial microbiome in asthmatic adults [24, 25], we recently showed similar findings in a murine model of steroid-resistant allergic asthma [85]. Intra-tracheal administration of fluticasone shifted the compositional structure of murine lung microbiota and intriguingly, also shifted that of the gut microbial community. The changes in gut microbiota composition were associated with increased representation of predicted bacterial metagenomic functions related to tryptophan metabolism, and in line with this, increased levels of kynurenine were measured in murine cecal contents post-fluticasone. The effects of pre-treatment with fluticasone on the murine microbiome also seem to augment negative outcomes from RSV infection in both allergen-sensitized and non-sensitized mice [86]. Specific mechanisms remain to be further elucidated, but altogether, these follow-up studies in murine models, along with in vitro studies of fluticasone effects on bacteria [84] lend further support to the biological plausibility of untoward effects of fluticasone on the airway microbiome and the latter's potential role in mediating therapeutic outcomes.

Additional examples of bedside-to-bench study of observed airway microbiome links to clinical outcomes come from recent investigations of bronchiectasis and COPD. For example, the ease of obtaining spontaneous expectorated sputum from bronchiectasis patients has allowed global cohorts to identify inflammatory endophenotypes distinguished by differences in combined microbial and cytokine mediator profiles [74, 99]. While certain airway pathogens are linked to worse bronchiectasis outcomes and can vary geographically, a recent study [100] examined how a highly understudied bacterium might shape airway biology and host response pathways in bronchiectasis. *Neisseria* spp. (e.g., *N. subflava*), which can be a challenge to cultivate, are understudied microbiota members that not infrequently have been reported to correlate with airway disease outcomes, including in asthma and chronic obstructive pulmonary disease [24, 25, 56, 97]. In their bronchiectasis cohort, Li et al. [100] showed that the presence of *Neisseria* spp. correlated with poor clinical outcomes and cultivated *Neisseria subflava* for further in vitro and in vivo work. The latter showed *N. subflava* promoting loss of epithelial integrity and inflammation in primary epithelial cells grown at air–liquid interface. In a murine model of *N. subflava* infection, metabolipidome analysis (lung and sera samples) indicated a proinflammatory environment induced by *N. subflava*, with some overlap with comparative human data. Altogether, results of this study suggest some *Neisseria* species, often overlooked as airway "commensals" may be pathobionts in chronic airway diseases. More research is broadly needed to understand what the enabling factors are (i.e., perturbations to an ecosystem) that allow an organism labeled as a pathobiont to assume more pathogen-like behaviors [101].

Similar veins of multi-omic studies coupled with bedside-to-bench translation are being pursued to dissect airway microbiome-host interactions in COPD [102–105], where again similar members of the airway microbiota have also been implicated in adult asthma. These studies also represent successes in overcoming the methodological challenges described earlier with airway samples for function-oriented microbiome characterization (e.g., use of shotgun metagenomics, metatranscriptomics and metabolomics). In one study, sputum microbiota analysis of a moderate to severe COPD cohort identified *Staphylococcus aureus* as one organism associated with

accelerated lung function decline [104]. Although additional organisms were also associated with rapid lung function decline, subsequent experimental work focused on understanding potential mechanisms involving *S. aureus*. Through a series of analyses and experiments, including metabolomic and genomic studies and use of an LPS/elastase murine model of emphysema, the investigators showed that airway colonization with *S. aureus* promoted lung function decline through homocysteine, which elicited a neutrophil apoptosis-to-NETosis shift via the AKT1-S100A8/A9 axis. Interestingly, using a bacteriophage targeting *S. aureus* to deplete the organism led to restored lung function in emphysematous mice [104]. Another study leveraged sputum microbiome data from a different moderate to severe COPD cohort and used the same experimental emphysema model to follow up a hypothesis uncovered from sputum multi-omic analyses of a role for altered tryptophan metabolism in the neutrophilic COPD phenotype [105]. Altered tryptophan metabolism was linked to reduced levels of indole-3-acetic acid (IAA) in the airway (indole derivatives being a product from bacterial metabolism of tryptophan). In vivo and in vitro studies showed that airway microbiota-derived IAA mitigated neutrophilic inflammation, apoptosis, emphysema, and lung function decline via macrophage–epithelial cell crosstalk mediated by interleukin-22. Intranasal inoculation of emphysematous mice with two airway lactobacilli restored IAA levels and demonstrated protective effects histologically and physiologically [105]. Thus, these two studies provide examples of promising avenues of new mechanistic insights into airway diseases informed by microbiome-integrated multi-omics.

Applying a reverse-translational paradigm [106] inclusive of multi-omics to the study of airway disease is thus strategic to advance biological and mechanistic insights informed by clinically meaningful observations. It is often stated and true that causal directionality cannot be firmly established from human studies; at the same time, unidirectional causality is arguably unlikely in chronic diseases. Even in lung diseases where a recognized pathogen or pathogenic community is involved (acute or chronic "infection"), evidence is also converging on the finding that outcomes in those diseases also vary in relation to and potentially depending on underlying differences in a specific individual's microbiome [107–109]. Microbiota are entwined with immune homeostasis and host responses that can result in a gradient of overall biological effect [110].

The functional basis for such in the respiratory tract and particularly in the lungs is only beginning to be understood. In asthma, the literature is also ripe with evidence on the role of the gut microbiota in early life, but it remains unknown to what extent and how gut microbiome functions in later life shape asthma outcomes or respiratory health. Moreover, across-compartment studies are lacking to glean the relative contributions of local (airways) versus systemic-derived (i.e., gut) microbiome influences on airways biology, including in asthma. As alluded to earlier, there is a crucial need for more "microbiome phenotyping," analogous for understanding the range of functional potential and mediators produced by airway microbiota in particular, that intersect with local immune responses. In this vein, understanding how airway microbiota and microbial metabolites might shape the functional phenotype of specific immune cell populations implicated in asthma would be especially interesting [111].

A recent joint workshop conducted by the American Thoracic Society and National Institute of Allergy and Infectious Diseases discussed additional gap areas and needs with respect to understanding the intersection of microbiome, metabolism, and immune regulation pathways in asthma [95].

Over the last decade, the field of respiratory microbiome research has blossomed, serendipitous with the rapid ascent of culture-independent tools to identify microbes in more difficult samples. With the ever-growing body of evidence implicating microbiota in airway disease heterogeneity, including in asthma, it is incumbent upon the research community to sustain this progress, which hopefully leads to novel strategies for more precise, potentially individualized interventions for asthma.

Acknowledgments

Y.J.H. did not receive specific funding support for this writing but acknowledges shared effort from the following NIH projects: NCATS U01TR004066, NHLBI 75N92024D00012-0-759202400001-1.

Conflicts of Interest

Y.J.H. has received research support for investigator-initiated studies from Regeneron and Sanofi that are managed by the University of Michigan. Dr. Huang also serves on the scientific advisory board for Alveolus Bio.

Data Availability Statement

The author has nothing to report.

References

1. A. Papi, C. Brightling, S. E. Pedersen, and H. K. Reddel, "Asthma," *Lancet* 391, no. 10122 (2018): 783–800.

2. W. C. Moore, D. A. Meyers, S. E. Wenzel, et al., "Identification of Asthma Phenotypes Using Cluster Analysis in the Severe Asthma Research Program," *American Journal of Respiratory and Critical Care Medicine* 181, no. 4 (2010): 315–323, https://doi.org/10.1164/rccm.200906-0896OC.

3. L. B. Bacharier, A. Beigelman, A. Calatroni, et al., "Longitudinal Phenotypes of Respiratory Health in a High-Risk Urban Birth Cohort," *American Journal of Respiratory and Critical Care Medicine* 199, no. 1 (2019): 71–82, https://doi.org/10.1164/rccm.201801-0190OC.

4. S. C. Dharmage, J. L. Perret, and A. Custovic, "Epidemiology of Asthma in Children and Adults," *Frontiers in Pediatrics* 7 (2019): 246.

5. M. J. Ege, M. Mayer, A. C. Normand, et al., "Exposure to Environmental Microorganisms and Childhood Asthma," *New England Journal of Medicine* 364, no. 8 (2011): 701–709, https://doi.org/10.1056/NEJMoa1007302.

6. M. G. Dominguez-Bello, F. Godoy-Vitorino, R. Knight, and M. J. Blaser, "Role of the Microbiome in Human Development," *Gut* 68, no. 6 (2019): 1108–1114.

7. O. Perdijk, R. Azzoni, and B. J. Marsland, "The Microbiome: An Integral Player in Immune Homeostasis and Inflammation in the Respiratory Tract," *Physiological Reviews* 104, no. 2 (2024): 835–879.

8. J. V. Fahy, "Type 2 Inflammation in Asthma—Present in Most, Absent in Many," *Nature Reviews. Immunology* 15, no. 1 (2015): 57–65.

9. K. F. Chung, "Asthma Phenotyping: A Necessity for Improved Therapeutic Precision and New Targeted Therapies," *Journal of Internal Medicine* 279, no. 2 (2016): 192–204.

10. A. M. Fitzpatrick, B. E. Chipps, F. Holguin, and P. G. Woodruff, "T2-"Low" Asthma: Overview and Management Strategies," *Journal of Allergy and Clinical Immunology. In Practice* 8, no. 2 (2020): 452–463.

11. S. Sharma, A. N. Gerber, M. Kraft, and S. E. Wenzel, "Asthma Pathogenesis: Phenotypes, Therapies, and Gaps: Summary of the Aspen Lung Conference 2023," *American Journal of Respiratory Cell and Molecular Biology* 71, no. 2 (2024): 154–168.

12. M. C. Arrieta, L. T. Stiemsma, P. A. Dimitriu, et al., "Early Infancy Microbial and Metabolic Alterations Affect Risk of Childhood Asthma," *Science Translational Medicine* 7, no. 307 (2015): 307ra152, https://doi.org/10.1126/scitranslmed.aab2271.

13. K. E. Fujimura, A. R. Sitarik, S. Havstad, et al., "Neonatal Gut Microbiota Associates With Childhood Multisensitized Atopy and T Cell Differentiation," *Nature Medicine* 22, no. 10 (2016): 1187–1191, https://doi.org/10.1038/nm.4176.

14. T. P. Wypych, C. Pattaroni, O. Perdijk, et al., "Microbial Metabolism of L-Tyrosine Protects Against Allergic Airway Inflammation," *Nature Immunology* 22, no. 3 (2021): 279–286.

15. J. Stokholm, M. J. Blaser, J. Thorsen, et al., "Maturation of the Gut Microbiome and Risk of Asthma in Childhood," *Nature Communications* 9, no. 1 (2018): 141, https://doi.org/10.1038/s41467-017-02573-2.

16. B. J. Marsland, A. Trompette, and E. S. Gollwitzer, "The Gut-Lung Axis in Respiratory Disease," *Annals of the American Thoracic Society* 12, no. Suppl 2 (2015): S150–S156.

17. S. D. Shukla, K. F. Budden, R. Neal, and P. M. Hansbro, "Microbiome Effects on Immunity, Health and Disease in the Lung," *Clinical & Translational Immunology* 6, no. 3 (2017): e133.

18. M. Özçam and S. V. Lynch, "The Gut-Airway Microbiome Axis in Health and Respiratory Diseases," *Nature Reviews. Microbiology* 22, no. 8 (2024): 492–506.

19. Y. J. Huang, C. E. Nelson, E. L. Brodie, et al., "Airway Microbiota and Bronchial Hyperresponsiveness in Patients With Suboptimally Controlled Asthma," *Journal of Allergy and Clinical Immunology* 127, no. 2 (2011): 372–381.

20. Y. J. Huang, S. Nariya, J. M. Harris, et al., "The Airway Microbiome in Patients With Severe Asthma: Associations With Disease Features and Severity," *Journal of Allergy and Clinical Immunology* 136, no. 4 (2015): 874–884, https://doi.org/10.1016/j.jaci.2015.05.044.

21. S. M. Teo, H. H. F. Tang, D. Mok, et al., "Airway Microbiota Dynamics Uncover a Critical Window for Interplay of Pathogenic Bacteria and Allergy in Childhood Respiratory Disease," *Cell Host & Microbe* 24, no. 3 (2018): 341–352.e5.

22. J. Thorsen, M. A. Rasmussen, J. Waage, et al., "Infant Airway Microbiota and Topical Immune Perturbations in the Origins of Childhood Asthma," *Nature Communications* 10, no. 1 (2019): 5001.

23. K. F. Chung, "Potential Role of the Lung Microbiome in Shaping Asthma Phenotypes," *Annals of the American Thoracic Society* 14, no. Supplement_5 (2017): S326–S331.

24. J. Durack, S. V. Lynch, S. Nariya, et al., "Features of the Bronchial Bacterial Microbiome Associated With Atopy, Asthma, and Responsiveness to Inhaled Corticosteroid Treatment," *Journal of Allergy and Clinical Immunology* 140, no. 1 (2017): 63–75.

25. J. Durack, L. S. Christian, S. Nariya, et al., "Distinct Associations of Sputum and Oral Microbiota With Atopic, Immunologic, and Clinical Features in Mild Asthma," *Journal of Allergy and Clinical Immunology* 146, no. 5 (2020): 1016–1026.

26. M. I. Abdel-Aziz, P. Brinkman, S. J. H. Vijverberg, et al., "Sputum Microbiome Profiles Identify Severe Asthma Phenotypes of Relative Stability at 12 to 18 Months," *Journal of Allergy and Clinical Immunology* 147, no. 1 (2021): 123–134.

27. X. Zhang, L. Li, J. Butcher, A. Stintzi, and D. Figeys, "Advancing Functional and Translational Microbiome Research Using Meta-Omics Approaches," *Microbiome* 7, no. 1 (2019): 154.

28. B. J. Didriksen, E. M. Eshleman, and T. Alenghat, "Epithelial Regulation of Microbiota-Immune Cell Dynamics," *Mucosal Immunology* 17, no. 2 (2024): 303–313.

29. C. M. Bassis, J. R. Erb-Downward, R. P. Dickson, et al., "Analysis of the Upper Respiratory Tract Microbiotas as the Source of the Lung and Gastric Microbiotas in Healthy Individuals," *MBio* 6, no. 2 (2015): e00037.

30. J. Durack, Y. J. Huang, S. Nariya, et al., "Bacterial Biogeography of Adult Airways in Atopic Asthma," *Microbiome* 6, no. 1 (2018): 104, https://doi.org/10.1186/s40168-018-0487-3.

31. H. Bisgaard, B. Chawes, J. Stokholm, M. Mikkelsen, A. M. M. Schoos, and K. Bønnelykke, "25 Years of Translational Research in the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC)," *Journal of Allergy and Clinical Immunology* 151, no. 3 (2023): 619–633.

32. K. McCauley, J. Durack, R. Valladares, et al., "Distinct Nasal Airway Bacterial Microbiotas Differentially Relate to Exacerbation in Pediatric Patients With Asthma," *Journal of Allergy and Clinical Immunology* 144, no. 5 (2019): 1187–1197.

33. K. E. McCauley, K. Flynn, A. Calatroni, et al., "Seasonal Airway Microbiome and Transcriptome Interactions Promote Childhood Asthma Exacerbations," *Journal of Allergy and Clinical Immunology* 150, no. 1 (2022): 204–213, https://doi.org/10.1016/j.jaci.2022.01.020.

34. S. M. Carney, J. C. Clemente, M. J. Cox, et al., "Methods in Lung Microbiome Research," *American Journal of Respiratory Cell and Molecular Biology* 62, no. 3 (2020): 283–299.

35. L. Tian, X. W. Wang, A. K. Wu, et al., "Deciphering Functional Redundancy in the Human Microbiome," *Nature Communications* 11, no. 1 (2020): 6217.

36. L. Li, T. Wang, Z. Ning, et al., "Revealing Proteome-Level Functional Redundancy in the Human Gut Microbiome Using Ultra-Deep Metaproteomics," *Nature Communications* 14, no. 1 (2023): 3428.

37. L. von Hertzen, M. J. Mäkelä, T. Petäys, et al., "Growing Disparities in Atopy Between the Finns and the Russians: A Comparison of 2 Generations," *Journal of Allergy and Clinical Immunology* 117, no. 1 (2006): 151–157.

38. M. Holbreich, J. Genuneit, J. Weber, C. Braun-Fahrländer, M. Waser, and E. von Mutius, "Amish Children Living in Northern Indiana Have a Very Low Prevalence of Allergic Sensitization," *Journal of Allergy and Clinical Immunology* 129, no. 6 (2012): 1671–1673, https://doi.org/10.1016/j.jaci.2012.03.016.

39. L. Ruokolainen, L. Paalanen, A. Karkman, et al., "Significant Disparities in Allergy Prevalence and Microbiota Between the Young People in Finnish and Russian Karelia," *Clinical and Experimental Allergy* 47, no. 5 (2017): 665–674, https://doi.org/10.1111/cea.12895.

40. P. V. Kirjavainen, A. M. Karvonen, R. I. Adams, et al., "Farm-Like Indoor Microbiota in Non-Farm Homes Protects Children From Asthma Development," *Nature Medicine* 25, no. 7 (2019): 1089–1095, https://doi.org/10.1038/s41591-019-0469-4.

41. C. Ober, A. I. Sperling, E. von Mutius, and D. Vercelli, "Immune Development and Environment: Lessons From Amish and Hutterite Children," *Current Opinion in Immunology* 48 (2017): 51–60.

42. G. Loss, S. Bitter, J. Wohlgensinger, et al., "Prenatal and Early-Life Exposures Alter Expression of Innate Immunity Genes: The PASTURE Cohort Study," *Journal of Allergy and Clinical Immunology* 130, no. 2 (2012): 523–530.e9.

43. B. Schaub, J. Liu, S. Höppler, et al., "Maternal Farm Exposure Modulates Neonatal Immune Mechanisms Through Regulatory T Cells," *Journal of Allergy and Clinical Immunology* 123, no. 4 (2009): 774–782.e5.

44. M. M. Stein, C. L. Hrusch, J. Gozdz, et al., "Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children," *New England Journal of Medicine* 375, no. 5 (2016): 411–421, https://doi.org/10.1056/NEJMoa1508749.

45. S. V. Lynch, R. A. Wood, H. Boushey, et al., "Effects of Early-Life Exposure to Allergens and Bacteria on Recurrent Wheeze and Atopy in Urban Children," *Journal of Allergy and Clinical Immunology* 134, no. 3 (2014): 593–601.e12.

46. A. Cait, M. R. Hughes, F. Antignano, et al., "Microbiome-Driven Allergic Lung Inflammation Is Ameliorated by Short-Chain Fatty Acids," *Mucosal Immunology* 11, no. 3 (2018): 785–795.

47. A. Trompette, E. S. Gollwitzer, K. Yadava, et al., "Gut Microbiota Metabolism of Dietary Fiber Influences Allergic Airway Disease and Hematopoiesis," *Nature Medicine* 20, no. 2 (2014): 159–166, https://doi.org/10.1038/nm.3444.

48. X. V. Li, I. Leonardi, and I. D. Iliev, "Gut Mycobiota in Immunity and Inflammatory Disease," *Immunity* 50, no. 6 (2019): 1365–1379.

49. X. Li, I. Leonardi, A. Semon, et al., "Response to Fungal Dysbiosis by Gut-Resident CX3CR1+ Mononuclear Phagocytes Aggravates Allergic Airway Disease," *Cell Host & Microbe* 24, no. 6 (2018): 847–856.e4.

50. A. N. Kanj, T. J. Kottom, K. J. Schaefbauer, M. Choudhury, A. H. Limper, and J. H. Skalski, "Dysbiosis of the Intestinal Fungal Microbiota Increases Lung Resident Group 2 Innate Lymphoid Cells and Is Associated With Enhanced Asthma Severity in Mice and Humans," *Respiratory Research* 24, no. 1 (2023): 144.

51. E. van Tilburg Bernardes, V. K. Pettersen, M. W. Gutierrez, et al., "Intestinal Fungi Are Causally Implicated in Microbiome Assembly and Immune Development in Mice," *Nature Communications* 11, no. 1 (2020): 2577, https://doi.org/10.1038/s41467-020-16431-1.

52. Y. H. Tsai, J. S. Parker, I. V. Yang, and S. N. P. Kelada, "Meta-Analysis of Airway Epithelium Gene Expression in Asthma," *European Respiratory Journal* 51, no. 5 (2018): 1701962.

53. Q. Zhang, M. Cox, Z. Liang, et al., "Airway Microbiota in Severe Asthma and Relationship to Asthma Severity and Phenotypes," *PLoS One* 11, no. 4 (2016): e0152724.

54. N. G. Papadopoulos, E. Apostolidou, M. Miligkos, and P. Xepapadaki, "Bacteria and Viruses and Their Role in the Preschool Wheeze to Asthma Transition," *Pediatric Allergy and Immunology* 35, no. 3 (2024): e14098.

55. A. A. T. M. Bosch, W. A. A. D. S. Piters, M. A. Van Houten, et al., "Maturation of the Infant Respiratory Microbiota, Environmental Drivers, and Health Consequences. A Prospective Cohort Study," *American Journal of Respiratory and Critical Care Medicine* 196, no. 12 (2017): 1582–1590.

56. Y. Zhou, D. Jackson, L. B. Bacharier, et al., "The Upper-Airway Microbiota and Loss of Asthma Control Among Asthmatic Children," *Nature Communications* 10, no. 1 (2019): 5714.

57. J. M. Mansbach, K. Hasegawa, P. A. Piedra, et al., "Haemophilus-Dominant Nasopharyngeal Microbiota Is Associated With Delayed Clearance of Respiratory Syncytial Virus in Infants Hospitalized for Bronchiolitis," *Journal of Infectious Diseases* 219, no. 11 (2019): 1804–1808.

58. K. M. Kloepfer, W. M. Lee, T. E. Pappas, et al., "Detection of Pathogenic Bacteria During Rhinovirus Infection Is Associated With Increased Respiratory Symptoms and Asthma Exacerbations," *Journal of Allergy and Clinical Immunology* 133, no. 5 (2014): 1301–1307.

59. A. Morin, C. G. McKennan, C. E. T. Pedersen, et al., "Epigenetic Landscape Links Upper Airway Microbiota in Infancy With Allergic Rhinitis at 6 Years of Age," *Journal of Allergy and Clinical Immunology* 146, no. 6 (2020): 1358–1366.

60. D. Rago, C. E. T. Pedersen, M. Huang, et al., "Characteristics and Mechanisms of a Sphingolipid-Associated Childhood Asthma Endotype," *American Journal of Respiratory and Critical Care Medicine* 203, no. 7 (2021): 853–863, https://doi.org/10.1164/rccm.202008-3206OC.

61. M. Rolando and C. Buchrieser, "A Comprehensive Review on the Manipulation of the Sphingolipid Pathway by Pathogenic Bacteria," *Frontiers in Cell and Development Biology* 7 (2019): 168.

62. S. L. Heaver, E. L. Johnson, and R. E. Ley, "Sphingolipids in Host-Microbial Interactions," *Current Opinion in Microbiology* 43 (2018): 92–99.

63. Y. Raita, C. A. Camargo, Y. A. Bochkov, et al., "Integrated-Omics Endotyping of Infants With Rhinovirus Bronchiolitis and Risk of Childhood Asthma," *Journal of Allergy and Clinical Immunology* 147, no. 6 (2021): 2108–2117, https://doi.org/10.1016/j.jaci.2020.11.002.

64. M. Fujiogi, Y. Raita, M. Pérez-Losada, et al., "Integrated Relationship of Nasopharyngeal Airway Host Response and Microbiome Associates With Bronchiolitis Severity," *Nature Communications* 13, no. 1 (2022): 4970.

65. N. Z. Kermani, C. Li, A. Versi, et al., "Endotypes of Severe Neutrophilic and Eosinophilic Asthma From Multi-Omics Integration of U-BIOPRED Sputum Samples," *Clinical and Translational Medicine* 14, no. 7 (2024): e1771.

66. A. J. Kozik, L. A. Begley, N. Lugogo, et al., "Airway Microbiota and Immune Mediator Relationships Differ in Obesity and Asthma," *Journal of Allergy and Clinical Immunology* 151, no. 4 (2023): 931–942.

67. D. E. Shaw, A. R. Sousa, S. J. Fowler, et al., "Clinical and Inflammatory Characteristics of the European U-BIOPRED Adult Severe Asthma Cohort," *European Respiratory Journal* 46, no. 5 (2015): 1308–1321, https://doi.org/10.1183/13993003.00779-2015.

68. R. Kaur and G. Chupp, "Phenotypes and Endotypes of Adult Asthma: Moving Toward Precision Medicine," *Journal of Allergy and Clinical Immunology* 144, no. 1 (2019): 1–12.

69. K. W. McGrath, N. Icitovic, H. A. Boushey, et al., "A Large Subgroup of Mild-To-Moderate Asthma Is Persistently Noneosinophilic," *American Journal of Respiratory and Critical Care Medicine* 185, no. 6 (2012): 612–619, https://doi.org/10.1164/rccm.201109-1640OC.

70. C. H. S. Kuo, S. Pavlidis, M. Loza, et al., "T-Helper Cell Type 2 (Th2) and Non-Th2 Molecular Phenotypes of Asthma Using Sputum Transcriptomics in U-BIOPRED," *European Respiratory Journal* 49, no. 2 (2017): 1602135.

71. J. G. Natalini, S. Singh, and L. N. Segal, "The Dynamic Lung Microbiome in Health and Disease," *Nature Reviews. Microbiology* 21, no. 4 (2023): 222–235.

72. M. I. Abdel-Aziz, S. J. H. Vijverberg, A. H. Neerincx, et al., "A Multi-Omics Approach to Delineate Sputum Microbiome-Associated Asthma Inflammatory Phenotypes," *European Respiratory Journal* 59, no. 1 (2022): 2102603.

73. K. Opron, L. A. Begley, J. R. Erb-Downward, et al., "Loss of Airway Phylogenetic Diversity Is Associated With Clinical and Pathobiological Markers of Disease Development in Chronic Obstructive Pulmonary Disease," *American Journal of Respiratory and Critical Care Medicine* 210, no. 2 (2024): 186–200, https://doi.org/10.1164/rccm.202303-0489OC.

74. Y. Gao, H. Richardson, A. J. Dicker, et al., "Endotypes of Exacerbation in Bronchiectasis: An Observational Cohort Study," *American Journal of Respiratory and Critical Care Medicine* 210, no. 1 (2024): 77–86, https://doi.org/10.1164/rccm.202310-1729OC.

75. E. R. Sutherland, T. S. King, N. Icitovic, et al., "A Trial of Clarithromycin for the Treatment of Suboptimally Controlled Asthma," *Journal of Allergy and Clinical Immunology* 126, no. 4 (2010): 747–753.

76. D. R. Denner, N. Sangwan, J. B. Becker, et al., "Corticosteroid Therapy and Airflow Obstruction Influence the Bronchial Microbiome, Which Is Distinct From That of Bronchoalveolar Lavage in Asthmatic Airways," *Journal of Allergy and Clinical Immunology* 137, no. 5 (2016): 1398–1405.e3.

77. S. Diver, K. Haldar, P. J. McDowell, et al., "Relationship Between Inflammatory Status and Microbial Composition in Severe Asthma and During Exacerbation," *Allergy* 77, no. 11 (2022): 3362–3376, https://doi.org/10.1111/all.15425.

78. J. R. Erb-Downward, D. L. Thompson, M. K. Han, et al., "Analysis of the Lung Microbiome in the "Healthy" Smoker and in COPD," *PLoS One* 6, no. 2 (2011): e16384.

79. A. A. Pragman, H. B. Kim, C. S. Reilly, C. Wendt, and R. E. Isaacson, "The Lung Microbiome in Moderate and Severe Chronic Obstructive Pulmonary Disease," *PLoS One* 7, no. 10 (2012): e47305.

80. M. A. Sze, P. A. Dimitriu, M. Suzuki, et al., "Host Response to the Lung Microbiome in Chronic Obstructive Pulmonary Disease," *American Journal of Respiratory and Critical Care Medicine* 192, no. 4 (2015): 438–445, https://doi.org/10.1164/rccm.201502-0223OC.

81. M. Garcia-Nuñez, L. Millares, X. Pomares, et al., "Severity-Related Changes of Bronchial Microbiome in Chronic Obstructive Pulmonary Disease," *Journal of Clinical Microbiology* 52, no. 12 (2014): 4217–4223, https://doi.org/10.1128/JCM.01967-14.

82. P. G. Woodruff, B. Modrek, D. F. Choy, et al., "T-Helper Type 2-Driven Inflammation Defines Major Subphenotypes of Asthma," *American Journal of Respiratory and Critical Care Medicine* 180, no. 5 (2009): 388–395, https://doi.org/10.1164/rccm.200903-0392OC.

83. N. R. Bhakta, O. D. Solberg, C. P. Nguyen, et al., "A qPCR-Based Metric of Th2 Airway Inflammation in Asthma," *Clinical and Translational Allergy* 3, no. 1 (2013): 24.

84. L. A. Begley, K. Opron, G. Bian, et al., "Effects of Fluticasone Propionate on Klebsiella Pneumoniae and Gram-Negative Bacteria Associated With Chronic Airway Disease," *mSphere* 7, no. 6 (2022): e0037722.

85. N. Asai, A. D. Ethridge, W. Fonseca, et al., "A Steroid-Resistant Cockroach Allergen Model Is Associated With Lung and Cecal Microbiome Changes," *Physiological Reports* 11, no. 13 (2023): e15761.

86. K. Yagi, A. D. Ethridge, N. R. Falkowski, et al., "Microbiome Modifications by Steroids During Viral Exacerbation of Asthma and in Healthy Mice," *American Journal of Physiology. Lung Cellular and Molecular Physiology* 327, no. 5 (2024): L646–L660, https://doi.org/10.1152/ajplung.00040.2024.

87. C. Crim, P. M. A. Calverley, J. A. Anderson, et al., "Pneumonia Risk in COPD Patients Receiving Inhaled Corticosteroids Alone or in Combination: TORCH Study Results," *European Respiratory Journal* 34, no. 3 (2009): 641–647.

88. S. Miethe, A. Karsonova, A. Karaulov, and H. Renz, "Obesity and Asthma," *Journal of Allergy and Clinical Immunology* 146, no. 4 (2020): 685–693.

89. A. E. Dixon and F. Holguin, "Diet and Metabolism in the Evolution of Asthma and Obesity," *Clinics in Chest Medicine* 40, no. 1 (2019): 97–106.

90. M. C. Peters and J. V. Fahy, "Metabolic Consequences of Obesity as an "Outside in" Mechanism of Disease Severity in Asthma," *European Respiratory Journal* 48, no. 2 (2016): 291–293.

91. V. A. Heinrich, C. Uvalle, M. L. Manni, et al., "Meta-Omics Profiling of the Gut-Lung Axis Illuminates Metabolic Networks and Host-Microbial Interactions Associated With Elevated Lung Elastance in a Murine Model of Obese Allergic Asthma," *Frontiers in Microbiomes* 2 (2023): 1153691, https://doi.org/10.3389/frmbi.2023.1153691.

92. Y. Liu, J. Zheng, H. P. Zhang, et al., "Obesity-Associated Metabolic Signatures Correlate to Clinical and Inflammatory Profiles of Asthma: A Pilot Study," *Allergy, Asthma & Immunology Research* 10, no. 6 (2018): 628–647.

93. M. C. Peters, K. W. McGrath, G. A. Hawkins, et al., "Plasma Interleukin-6 Concentrations, Metabolic Dysfunction, and Asthma Severity: A Cross-Sectional Analysis of Two Cohorts," *Lancet Respiratory Medicine* 4, no. 7 (2016): 574–584, https://doi.org/10.1016/S2213-2600(16)30048-0.

94. S. Singh, J. G. Natalini, and L. N. Segal, "Lung Microbial-Host Interface Through the Lens of Multi-Omics," *Mucosal Immunology* 15, no. 5 (2022): 837–845.

95. A. J. Kozik, F. Holguin, L. N. Segal, et al., "Microbiome, Metabolism, and Immunoregulation of Asthma: An American Thoracic Society and National Institute of Allergy and Infectious Diseases Workshop Report," *American Journal of Respiratory Cell and Molecular Biology* 67, no. 2 (2022): 155–163.

96. K. L. Bowerman, S. F. Rehman, A. Vaughan, et al., "Disease-Associated Gut Microbiome and Metabolome Changes in Patients With Chronic Obstructive Pulmonary Disease," *Nature Communications* 11, no. 1 (2020): 5886.

97. S. S. Madapoosi, C. Cruickshank-Quinn, K. Opron, et al., "Lung Microbiota and Metabolites Collectively Associate With Clinical Outcomes in Milder Stage Chronic Obstructive Pulmonary Disease," *American Journal of Respiratory and Critical Care Medicine* 206, no. 4 (2022): 427–439, https://doi.org/10.1164/rccm.202110-2241OC.

98. M. Y. Ramsheh, K. Haldar, A. Esteve-Codina, et al., "Lung Microbiome Composition and Bronchial Epithelial Gene Expression in Patients With COPD Versus Healthy Individuals: A Bacterial 16S rRNA Gene Sequencing and Host Transcriptomic Analysis," *Lancet Microbe* 2, no. 7 (2021): e300–e310.

99. H. Choi, S. Ryu, H. R. Keir, et al., "Inflammatory Molecular Endotypes in Bronchiectasis: A European Multicenter Cohort Study," *American Journal of Respiratory and Critical Care Medicine* 208, no. 11 (2023): 1166–1176, https://doi.org/10.1164/rccm.202303-0499OC.

100. L. Li, M. Mac Aogáin, T. Xu, et al., "Neisseria Species as Pathobionts in Bronchiectasis," *Cell Host & Microbe* 30, no. 9 (2022): 1311–1327.e8.

101. L. Jochum and B. Stecher, "Label or Concept—What Is a Pathobiont?," *Trends in Microbiology* 28, no. 10 (2020): 789–792.

102. I. Sulaiman, B. G. Wu, Y. Li, et al., "Functional Lower Airways Genomic Profiling of the Microbiome to Capture Active Microbial Metabolism," *European Respiratory Journal* 58, no. 1 (2021): 2003434, https://doi.org/10.1183/13993003.03434-2020.

103. Z. Wang, Y. Yang, Z. Yan, et al., "Multi-Omic Meta-Analysis Identifies Functional Signatures of Airway Microbiome in Chronic Obstructive Pulmonary Disease," *ISME Journal* 14, no. 11 (2020): 2748–2765.

104. W. Liang, Y. Yang, S. Gong, et al., "Airway Dysbiosis Accelerates Lung Function Decline in Chronic Obstructive Pulmonary Disease," *Cell Host & Microbe* 31, no. 6 (2023): 1054–1070.e9.

105. Z. Yan, B. Chen, Y. Yang, et al., "Multi-Omics Analyses of Airway Host–Microbe Interactions in Chronic Obstructive Pulmonary Disease Identify Potential Therapeutic Interventions," *Nature Microbiology* 7, no. 9 (2022): 1361–1375.

106. V. Shakhnovich, "It's Time to Reverse Our Thinking: The Reverse Translation Research Paradigm," *Clinical and Translational Science* 11, no. 2 (2018): 98–99, https://doi.org/10.1111/cts.12538.

107. G. Mori, M. Morrison, and A. Blumenthal, "Microbiome-Immune Interactions in Tuberculosis," *PLoS Pathogens* 17, no. 4 (2021): e1009377.

108. G. D. Kitsios, K. Sayed, A. Fitch, et al., "Longitudinal Multicompartment Characterization of Host-Microbiota Interactions in Patients With Acute Respiratory Failure," *Nature Communications* 15, no. 1 (2024): 4708, https://doi.org/10.1038/s41467-024-48819-8.

109. S. Widder, L. A. Carmody, K. Opron, L. M. Kalikin, L. J. Caverly, and J. J. LiPuma, "Microbial Community Organization Designates Distinct Pulmonary Exacerbation Types and Predicts Treatment Outcome in Cystic Fibrosis," *Nature Communications* 15, no. 1 (2024): 4889.

110. A. Casadevall and L. A. Pirofski, "What Is a Host? Incorporating the Microbiota Into the Damage–Response Framework," *Infection and Immunity* 83, no. 1 (2015): 2–7.

111. C. N. Castro, J. Freitag, L. Berod, M. Lochner, and T. Sparwasser, "Microbe-Associated Immunomodulatory Metabolites: Influence on T Cell Fate and Function," *Molecular Immunology* 68, no. 2 Pt C (2015): 575–584.