

The respiratory microbiome in childhood asthma



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Asthma is the most prevalent noncommunicable disease in childhood, characterized by reversible airway constriction and inflammation of the lower airways. The respiratory tract consists of the upper and lower airways, which are lined with a diverse community of microbes. The composition and density of the respiratory microbiome differs across the respiratory tract, with microbes adapting to the gradually changing physiology of the environment. Over the past decade, both the upper and lower respiratory microbiomes have been implicated in the etiology and disease course of asthma, as well as in its severity and phenotype. We have reviewed the literature on the role of the respiratory microbiome in asthma, making a careful distinction between the relationship of the microbiome with development of childhood asthma and its relationship with the disease course, while accounting for age and the microbial niches studied. Furthermore, we have assessed the literature regarding the underlying asthma endotypes and the impact of the microbiome on the host immune response. We have identified distinct microbial signatures across the respiratory tract associated with asthma development, stability, and severity. These data suggest that the respiratory microbiome may be important for asthma development and severity and may therefore be a potential target for future microbiome-based preventive and treatment strategies. (*J Allergy Clin Immunol* 2023;152:1352-67.)

Key words: Asthma, wheeze, respiratory microbiome, pediatric

Asthma is a heterogeneous disease characterized by chronic airway inflammation and variable limitation of expiratory airflow.¹ It is the most common chronic noncommunicable disease in childhood, and it can be classified on the basis of clinical

Abbreviations used

COPD: Chronic obstructive pulmonary disease
OCS: Oral corticosteroid
RSV: Respiratory syncytial virus
RTI: Respiratory tract infection
RV: Rhinovirus
RV-C: Rhinovirus type C
Treg: Regulatory T
TSLP: Thymic stromal lymphopoietin

traits (phenotypes), including age of onset and family history, or alternatively, underlying immune pathways referred to as endotypes. These asthma endotypes are broadly categorized as T_H2 cell-high and T_H2 cell-low asthma endotypes.^{2,3} Although the exact etiology of asthma development is not fully understood, it is influenced by the interplay between genetic and environmental factors, including the respiratory microbiome, which entails all microorganisms inhabiting the respiratory tract.

The respiratory tract consists of the upper and lower airways, each containing smaller anatomic structures or “niches.” The upper airways include the nasopharynx, oropharynx, (hypo) pharynx, and larynx, as well as the lower airways, trachea, bronchioles, and alveoli. The various anatomic structures within the respiratory system differ in physiologic properties, including the type of cell lining, pH, humidity, temperature, mucus production, and oxygen and carbon dioxide pressure. Although the airways constitute a single organ system and form a microbial continuum, the microorganisms inhabiting the airways differ across niches owing to these variable physiologic conditions.⁴⁻⁶ Hence, it is not surprising that although asthma mainly affects the lower airways, studies have shown altered microbiome compositions throughout the entire respiratory system.^{7,8} This underlines their interdependency and demonstrates a need to study the respiratory microbiome in a more holistic manner.

A growing body of evidence associates the respiratory microbiome with both asthma etiology⁹⁻¹¹ and disease trajectory. With regard to the latter, studies have investigated the respiratory microbiome in asthmatic individuals versus in healthy controls,¹² as well as in relation to asthma severity,¹³ symptom progression, and exacerbations.^{14,15} Still, studies of the respiratory microbiome in relation to asthma are heterogeneous in design, target different respiratory niches, and/or include patients with different asthma phenotypes or endotypes. Consequently, distilling definitive conclusions regarding the associations between the respiratory microbiome and asthma is difficult. In this review, we have aimed to comprehensively summarize the existing literature on microbiome-based studies in childhood asthma and provide

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results in the context of asthma development, disease trajectory, and underlying immune responses associated with asthma while considering natural age-related changes in the microbiome and anatomic differences. We performed a systematic search for asthma or wheeze, respiratory microbiome, development, and severity (see [Box 1](#), which details the search strategy and selection criteria). Briefly, we selected studies examining the bacterial respiratory microbiome in children and excluded studies focusing exclusively on the mycobiome, virome, or gastrointestinal microbiome.

THE RESPIRATORY MICROBIOTA AND ASTHMA DEVELOPMENT

Microbial colonization of a newborn starts during birth,^{16,17} following the rupture of membranes and passage through the maternal birth canal. Cesarean sections can lead to aberrant early-life colonization, where the first microbial exposure originates from the maternal skin and the hospital environment. Perinatal antibiotic exposure or a lack of breast-feeding have also been associated with altered early-life development of the microbiome. Altered early-life microbiota development has in turn been related to the development of wheeze and/or asthma later in childhood ([Fig 1](#) and [Table 1](#)^{9-11,18-30}). Several population-based birth cohort studies have described associations between early-life respiratory microbiota and the development of transient, recurrent, and persistent wheeze or asthma.^{11,18,28} They found that higher bacterial diversity and abundances of *Veillonella* and *Prevotella* in the hypopharynx at age 1 month were associated with asthma diagnoses at the age of 6 years.¹¹ Subsequently, an increase in nasal *Haemophilus* abundance and persistent low abundance of *Moraxella* over the first year of life have been associated with physician-diagnosed asthma at age 7 years.¹⁸ In contrast, high abundance of nasal *Lactobacillus* at 2 months of age, gradually decreasing in abundance over the next year,¹⁸ and high abundance of oropharyngeal *Lactobacillus* at age 2 years²⁹ have been associated with a lower risk of asthma development at age 7 years.^{18,29} Another study found that high levels of *Granulicatella* at the end of the first year of life and *Prevotella* after 1.5 years of life were associated with a lower risk of development of wheeze by age 2 years, whereas an increase in level of oropharyngeal *Neisseria* over the second year of life was positively associated with the development of wheeze.²⁸

Various studies of the respiratory microbiome and asthma development included children with a parental history of asthma, eczema, hay fever, and/or allergic rhinitis.^{9,10,21,22} In a first culture-based study, hypopharyngeal colonization with *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* at age 1 month, but not *Staphylococcus aureus*, was associated with asthma development at age 5 years.⁹ Conversely, another study found that *Staphylococcus*-dominant nasopharyngeal profiles in the first half year of life were associated with recurrent wheeze in the first 3 years of life and subsequent physician-diagnosed asthma at ages 6, 8, 11, 13, and 18 years.²¹ The discrepancy between these findings may be due to differences in participant ages or the distinct ecologic roles of *Staphylococcus* species in each respiratory niche (hypopharynx versus nasopharynx). Consistent with the culture-based study, a culture-independent study also found that higher nasopharyngeal abundance of *Streptococcus* in the first 2 months of life was associated with later asthma development.¹⁰ Not only early-life

colonization but also the frequency of having a *Streptococcus*, *Haemophilus*, or *Moraxella*-dominated nasopharyngeal profile at ages 2, 6, 12, 18, and 24 months was associated with an increased risk of chronic wheeze at age 5 years in early-sensitized children (defined as any allergen-specific IgE level > 0.35kU/L at age 2 years), whereas for nonsensitized children the association was apparent only for wheezing episodes in the first 3 years of life.²²

Certain early-life microbiota signatures have also been associated with development of recurrent respiratory tract infections (RTIs) in early life. For example, profiles dominated by *Streptococcus* and *Moraxella* have been shown to be attributed to the timing and recurrence of the first RTI,^{10,31} whereas *Corynebacterium*, *Alloiococcus*, and *Staphylococcus* were negatively associated with (lower) RTIs.²² Furthermore, earlier timing and severity of RTIs, including lower RTIs with fever, wheeze, and rhinovirus (RV) type C (RV-C), have been associated with an increased risk of chronic wheeze at age 5 years.¹⁰ Interestingly, composition of the microbial community at the time of these RTIs, especially the interplay between bacteria and viruses, has been associated with the risk of wheeze and asthma development later in life. Consistent with findings in times of health, higher abundances of *S pneumoniae*, *Moraxella* (*M catarrhalis*), and *Haemophilus* (*H influenzae*) at the time of a viral RTI (especially RV-C or respiratory syncytial virus [RSV] infection) are associated with the risk of developing recurrent wheeze^{23,30} and asthma.^{21,24-27} These studies suggest a temporal correlation and interplay between the respiratory microbiome, infection risk, infection severity, and the development of wheeze and asthma. Further supporting this theory, increased levels of nasopharyngeal *Moraxella* and *Streptococcus* after recovery of an early-life RTI have been associated with recurrent wheeze and asthma later in life.¹⁹ Additionally, higher abundance of nasal *Lactobacillus* at the time of an RTI is also associated with a lower risk of developing recurrent wheeze,²⁰ again underlining this temporal correlation between the respiratory microbiota at the time of an RTI and development of wheeze. [Table 1](#) summarizes the main findings of studies of the bacterial respiratory microbiota in relation to wheeze and asthma development, stratified by niche. Altogether, multiple studies have shown an association between early-life *Streptococcus* (*S pneumoniae*), *Moraxella* (*M catarrhalis*), and *Haemophilus* (*H influenzae*)^{9,10,18,22,23,30} and wheeze and/or asthma development. In contrast, high early-life levels of *Lactobacillus* in the nasal cavity^{18,20} and oropharynx²⁹ are associated with lower risk of developing wheeze and/or asthma, both during periods of health^{18,29} and during an RTI.²⁰ In conclusion, the data on early-life respiratory microbiota and future asthma development show a recurrent microbial signature associated with early-life respiratory infection and consecutive wheeze and/or asthma development.

THE RESPIRATORY MICROBIOTA AND THE DISEASE TRAJECTORY

The respiratory microbiota in acute wheeze

At the time of acute wheeze, the respiratory microbiota differs from that of children without wheeze ([Table II](#)^{12-15,21,29,32-48} and [Fig 2](#)). Again, *Haemophilus* and *Moraxella* levels are higher in children with wheeze, along with elevated *Neisseria* levels compared with the levels in healthy controls. These associations have been observed throughout the upper respiratory tract. The

Box 1. Search strategy and selection criteria

Search strategy

- We identified relevant articles by searching PubMed and the reference list of selected articles. Titles and abstracts of articles were screened for relevance.
- We searched for papers on respiratory microbiota (search terms “microbiome,” “microbiota,” “bacteria,” “respiratory,” “upper respiratory tract,” “lower respiratory tract,” “airway,” “nasopharynx,” “nasal,” “oropharynx”), wheeze and/or asthma development (search terms “wheeze,” “asthma,” “development”), respiratory microbiota and severity (search terms “wheeze,” “asthma,” “severe,” “therapy resistant”), and respiratory microbiota and wheeze and/or asthma exacerbation (search terms “wheeze,” “asthma,” “exacerbation,” “attack”).

Inclusion criteria

- Article is written in English.
- Results include data on pediatric subjects.
- Authors studied the respiratory microbiota.
- Asthma or wheeze was 1 of the diseases studied.

Exclusion criteria

- Study characterizes only the virome or mycobiome.
- Study focuses on the gut microbiome.
- Study focuses on allergic outcomes without asthma.

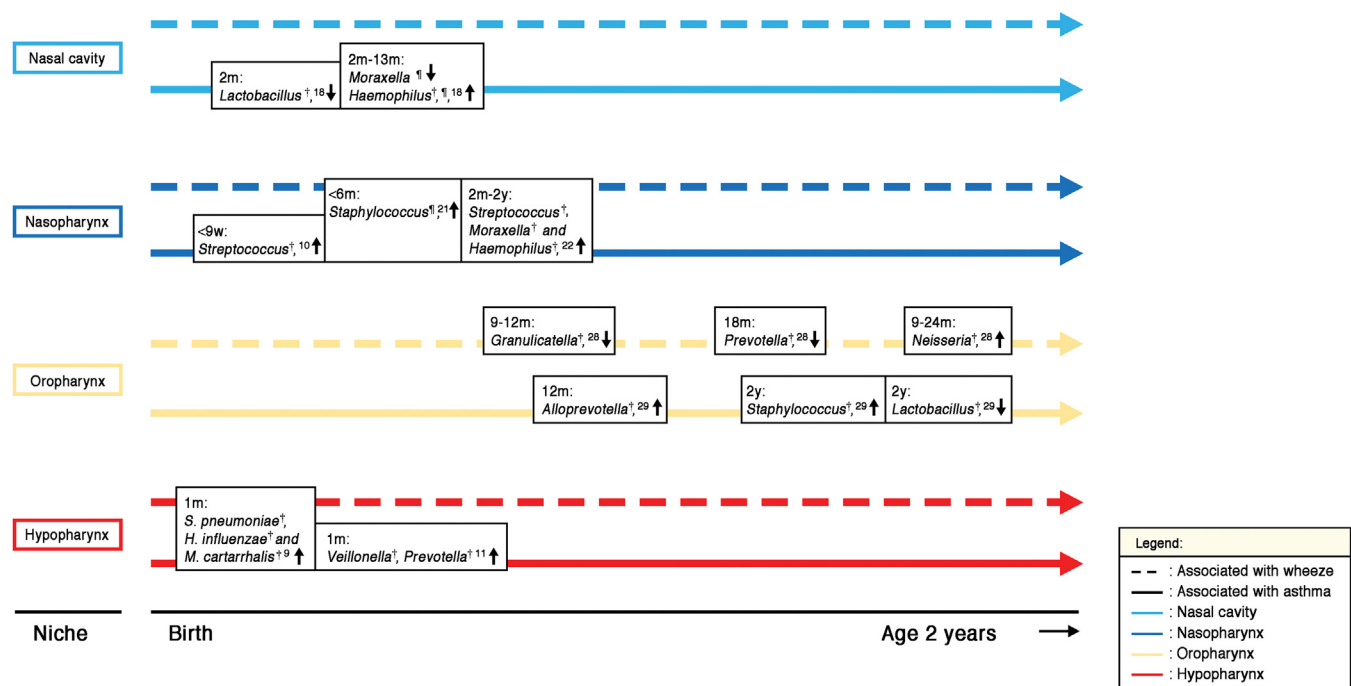


FIG 1. Respiratory microbiota signals in early life related to consecutive development of asthma and/or wheeze. X-axis represents the interval from birth until the second year, before future wheeze and/or asthma onset (right arrow). Upward arrow in boxes indicates a positive association between early life respiratory microbiota and subsequent development of (future) wheeze and/or asthma (dashed line and straight line, respectively), whereas downward arrow represents a negative association. Lines are colored per respiratory niche. ¶Microbiome profile. †Genus or species (relative) abundance.

first, culture-based study, showed that *H influenzae* and *M catarrhalis* were cultured more frequently from the hypopharynx of children experiencing wheezing episodes in the first 3 years of life.³⁵ A second study, which compared children with acute wheeze, children with acute asthma, and healthy controls (mean age 2.3, 10.3, and 8.5 respectively), showed higher relative abundances of *Moraxella* and *Haemophilus* in the nasopharynx of children with acute wheeze,³³ although the significant age difference

could partially account for this effect. A third, smaller study showed a correlation between higher relative abundance of oropharyngeal *Neisseria elongate* and recurrent wheeze.³⁴ Finally, 1 article showed that children with *Haemophilus*-, *Neisseria*-, or *Streptococcus*-dominated hypopharyngeal microbiota or with a high relative abundance of *Veillonella* had significantly longer wheezing episodes than children with a mixed microbiota profile did.³⁶ One study found nasopharyngeal *S pneumoniae* to

TABLE I. The respiratory microbiota and asthma development

Study and niche	No. and type of participants	Definition of asthma-related outcome	Main findings of interest
Toivonen et al ¹⁸ NC	n = 704; followed to age 7 y	Asthma: physician-diagnosed asthma	↓ <i>Moraxella</i> * in year 1 of life → higher risk of asthma ↑ <i>Haemophilus</i> † in year 1 of life → higher risk of asthma ↑ <i>Lactobacillus</i> † at 2m → lower risk of asthma
Mansbach et al ¹⁹ NC	n = 842; children hospitalized with bronchiolitis at age <1 y; followed to age 4 y	Recurrent wheeze at age 3 y: ≥2 steroid courses for exacerbations lasting <6 mo or ≥4 wheezing episodes in past 12 mo Asthma at age 4 y: physician-diagnosed asthma and asthma medication or asthma related symptoms between ages 3 and 4 y	↑ <i>Moraxella</i> † and <i>Streptococcus</i> † 3 wk after hospitalization → higher risk of recurrent wheeze and asthma ↑ <i>Streptococcus</i> † in summer after hospitalization → higher risk of recurrent wheeze
Rosas-Salazar et al ²⁰ NC	n = 118; children with RSV-positive ARI at age ≤1 y; followed to age 2 y	Subsequent wheeze: parental report of wheeze since last birthday Recurrent wheeze: parental report of ≥2 episodes of wheeze since last birthday	↑ <i>Lactobacillus</i> † during ARI → lower risk of subsequent and recurrent wheeze
Tang et al ²¹ NP	n = 289; children who had ≥1 parent with a history of asthma; followed to age 18 y	Wheezing illness: ≥1 of the following: (1) physician-diagnosed wheeze; (2) prescribed SABA, LABA, and/or long-term controller medication; and (3) diagnosed bronchiolitis, wheezing illness, reactive airway disease, asthma, or asthma exacerbation Asthma at age ≥6 y: ≥1 of the following: (1) physician-diagnosed asthma, (2) physician prescribed albuterol, (3) daily controller medication, (4) rescue medication with albuterol or ICS, and (5) prednisone for asthma exacerbation	↑ <i>Staphylococcus</i> * in first 6 mo of life → higher risk of recurrent wheeze at age 3 y and asthma from age 6-18 y ↑ RV + <i>Moraxella</i> * → higher risk at asthma from age 6-18 y
Teo et al ¹⁰ NP	n = 234; children who had ≥1 parent with physician-diagnosed asthma, hay fever, or eczema; followed to age 10 y	Current wheeze: parent-reported wheeze 12 mo before 5-y or 10-y visit	↑ <i>Streptococcus</i> † at age <9 wk → higher risk of wheeze at ages 5 y and 10 y ↑ <i>Streptococcus</i> , <i>Moraxella</i> * → early respiratory infection → higher risk of wheeze at age 5 y ↑ <i>Streptococcus</i> , <i>Moraxella</i> , <i>Haemophilus</i> * during ARI → (febrile) LRI in y 1 of life → higher risk of wheeze at age 5 y RV-C LRI with wheezing → higher risk at chronic wheeze
Teo et al ²² NP	n = 244; children who had ≥1 parent with physician-diagnosed asthma, hay fever, or eczema; followed to age 5 y	Sensitization: laboratory-confirmed Wheeze at age 5 y: parent-reported wheeze 12 mo before 5-y visit Transient wheeze: parent reported wheezing episodes in only ≤3 y of life Early allergic sensitization: laboratory-confirmed sensitization by age 2 y	↑ <i>Streptococcus</i> , <i>Haemophilus</i> , <i>Moraxella</i> * at ages 2 mo, 6 mo, 12 mo, 18 mo, 24 mo + early positive RAST result → higher risk of chronic wheeze at age 5 y ↑ <i>Streptococcus</i> , <i>Haemophilus</i> , <i>Moraxella</i> * at ages 2, 6, 12, 18, and 24 mo + negative RAST result → higher risk of transient wheeze in nonsensitized children
Dumas et al ²³ NP	n = 921; children hospitalized with bronchiolitis at age <1 y; followed until age 3 y	Bronchiolitis: physician-diagnosed recurrent wheeze at 3 y: parental report of ≥2 corticosteroid-requiring breathing problems in 6 mo or ≥4 breathing problem episodes in 1 y that last ≥1 d and affect sleep Asthma at age 3 y: parent-reported asthma	<i>Haemophilus</i> and <i>Moraxella</i> * + non-RSV (mostly RV) infection + history of breathing problems and eczema + older age at bronchiolitis hospitalization → higher risk of recurrent wheeze and asthma at age 3 y

(Continued)

TABLE I. (Continued)

Study and niche	No. and type of participants	Definition of asthma-related outcome	Main findings of interest
Raita et al ²⁴ NP	n = 122; children hospitalized at age ≤1 y with RV-positive bronchiolitis; followed to age 5 y	Recurrent wheeze at age 3 y: ≥2 exacerbations with corticosteroid in 6 mo or ≥4 wheeze episodes in 1 y that last ≥1 d and affect sleep Asthma at age 5 y: physician-diagnosed asthma at age 5 y and use of asthma medication or asthma-related symptoms in preceding year	<i>Moraxella</i> * + RV-C infection + high type 2 cytokine levels → higher risk of recurrent wheeze and asthma
Raita et al ²⁵ NP	n = 221; children hospitalized at age ≤1 y with RSV-positive bronchiolitis; followed to age 5 y	Recurrent wheeze at age 3 y: ≥2 exacerbations with corticosteroids in 6 mo or ≥4 wheezing episodes in 1 y that last ≥1 d and affect sleep Asthma at age 5 y: physician-diagnosed asthma at age 5 y and use of asthma medication (albuterol, ICS, montelukast) or asthma-related symptoms in preceding year	<i>S pneumoniae</i> / <i>M catarrhalis</i> * + RSV/RV coinfection + high IgE sensitization + high IFN-α and IFN-γ levels → higher risk of recurrent wheeze and asthma
Raita et al ²⁶ NP	n = 244; children hospitalized with bronchiolitis at age ≤1 y; followed to age 5 y	Asthma at age 5 y: physician-diagnosed asthma at age 5 y and use of asthma medication (albuterol, ICS, montelukast) or asthma-related symptoms in preceding year	<i>H influenzae</i> - or <i>S pneumoniae</i> -* dominated microbiota + RSV or RSV/RV-A infection → higher risk of asthma
Zhu et al ²⁷ NP	n = 244; children hospitalized with bronchiolitis at age ≤1 y; followed to age 6 y	Asthma at age 6 y: physician-diagnosed asthma at age 6 y and use of asthma medication (albuterol, ICS, montelukast) or asthma-related symptoms in the preceding year	↑ <i>S pneumoniae</i> † → Higher risk of asthma (particularly in infants with non-RV infection)
Powell et al ²⁸ OP	n = 159; followed to age 2 y	Physician-diagnosed wheeze: physician-diagnosed wheeze or prescription of bronchodilator in records Recurrent wheeze: ≥1 episode of physician-diagnosed wheeze at age 2 y	↑ <i>Neisseria</i> † levels at age 18 mo and between ages 9 and 24 mo → higher risk of wheeze ↑ <i>Granulicatella</i> † between ages 9 and 12 mo and <i>Prevotella</i> † at age ≥18 mo → lower risk of wheeze
Dzidic et al ²⁹ OP	n = 80; RCT for <i>Lactobacillus reuteri</i> ; with allergy (n = 47); with asthma (n = 20); healthy controls (n = 33); followed from age 3 mo to age 7 y	Asthma: ≥1 of the following: (1) physician-diagnosis and asthma symptoms and/or medication at age <12 mo and (2) wheeze or nocturnal cough and positive reversibility test result with spirometry at age 7 y	↑ <i>Alloprevotella</i> † at age 12 mo and <i>Staphylococcus</i> † at age 24 mo → higher risk at asthma ↓ <i>Lactobacillus crispatus</i> † and <i>Lactobacillus gasseri</i> † over the first 7 y of life (at ages 3, 6, 12, and 24 mo and 7 y) and <i>Atopobium</i> † at age 24 mo → higher risk at asthma
Bisgaard et al ⁹ HP	n = 321; children from mothers with history of asthma; followed to age 5 y	Persistent wheeze: 5 wheeze episodes in 6 mo or daily symptoms for 4 wk and exclusion of other diagnoses Exacerbation of wheeze: physician-diagnosed and treatment with oral or high-dose corticosteroids Asthma at age 5 y: physician-diagnosed asthma based on (1) history of persistent symptoms, (2) response to 3-mo course of ICSs, (3) need for intermittent rescue medication (bronchodilators) 12 mo before age 5 y	<i>S pneumoniae</i> ,† <i>H influenzae</i> ,† and <i>M catarrhalis</i> † at age 1 mo → higher risk of persistent wheeze, (hospitalization for) exacerbation of wheeze and eventually asthma
Thorsen et al ¹¹ HP	n = 695; followed from age 24 wk to 6 y	Asthma: symptom relapse after cessation of ICSs, given for 3 mo when parents reported (1) ≥5 episodes lasting ≥3 d within 6 mo, (2) 4 wk of continuous symptoms, (3) any acute severe episode necessitating hospitalization, or (4) treatment with OCSs	↑ α-Diversity, <i>Veillonella</i> † and <i>Prevotella</i> † at age 1 mo → asthma in first 6 y of life

(Continued)

TABLE I. (Continued)

Study and niche	No. and type of participants	Definition of asthma-related outcome	Main findings of interest
Zhang et al ³⁰ Lower airways‡	n = 74; Children aged <6 mo, with RSV-positive wheezing bronchiolitis; followed to age 3 y	Severe bronchiolitis: requiring oxygen supplement and hospital admission Wheezing episode: parent-reported wheeze with an ICS or hospitalization in records Recurrent wheeze: ≥3 episodes of pediatrician-diagnosed wheeze	↑ <i>Haemophilus</i> †, <i>Moraxella</i> †, <i>Klebsiella</i> † → higher risk of recurrent wheeze

ARI, Acute respiratory infection; HP, hypopharynx; ICS, inhalation corticosteroid; IFN, interferon; LABA, long-acting β -agonist; LRI, lower respiratory infection; NC, nasal cavity; NP, nasopharynx; OP, oropharynx; RAST, radioallergosorbent test; RCT, randomized controlled trial; SABA, short-acting β -agonist.

*Microbiome profile.

†Genus or species (relative) abundance.

‡Lower airways sampled through nasotracheal aspirates.

be associated with acute exacerbated wheeze in first 3 years of life.²¹ In contrast, healthy controls had higher levels of oropharyngeal *Eubacterium sulci*, *Leptotrichia wadei*, and *Prevotella* spp than in children with recurrent wheeze in the previous year³⁴ and higher levels of *Dolosigranulum (D pigrum)* than in children with acute exacerbated wheeze.^{21,33} In summary, the literature shows consistent ecologic differences between children with wheeze and healthy controls, with higher abundances of *Haemophilus*, *Moraxella*, and *Neisseria* being associated with the presence and duration of wheeze.

The respiratory microbiota in asthma

The respiratory microbiota in children with asthma, which is typically diagnosed at the age of 5 years or older,¹ also differs from that of healthy controls (Table II and Fig 2). As with respiratory microbiota in wheeze, children with asthma have higher *Moraxella* levels than healthy controls do. This is supported by a large study in 327 children, aged 6 to 12 years, that found higher *Moraxella* levels in the nasal cavity of non-farm-living children with asthma (but not in those living on a farm) than in healthy controls of the same age.¹² This implies that the microbiota may be a mediator, linking environmental exposures and subsequent asthma development. Two smaller studies also found differences in the lower airway microbiota of children with asthma and that of healthy controls,^{41,42} demonstrating elevated levels of *Moraxella* and *Streptococcus*.⁴² Although some studies have found differences in oropharyngeal microbiota, including higher levels of *Haemophilus*³⁸ and *Veillonella*³⁹ and lower levels of *Streptococcus*,^{29,39} *Fusobacterium*, *Capnocytophaga*, and *Lactobacillus*,²⁹ these differences were not consistently reported in all studies characterizing this niche in asthma.^{12,41} This suggests that differences in oropharyngeal microbiome composition may be less pronounced in the context of asthma.

Some children with asthma experience uncontrolled symptoms and/or frequent exacerbations requiring oral corticosteroids (OCSs) and/or hospitalization.¹ A subset of this group, which continues to experience uncontrolled asthma despite treatment with a medium- or high-dose inhalation corticosteroid combined with a second controller or maintenance OCS, is referred to as difficult-to-treat, therapy-resistant, or severely asthmatic.¹ The respiratory microbiome of these children also differs from that of healthy children (Table II and Fig 2).^{13,37} The first study to describe these differences found higher abundances of *Haemophilus* and *Staphylococcus* in the lower airways,¹³ and another found higher levels of *Streptococcus* in the nasal cavity.³⁷

Interestingly, neither of these studies^{13,37} describe differences in *Moraxella* levels between children with severe asthma and healthy controls, whereas this was observed in children with stable asthma for the same respiratory niches.^{12,42} The studies that investigated the respiratory microbiota in severe asthma had small sample sizes, potentially limiting their ability to detect significant shifts in the relative abundance of commensals such as *Moraxella*. Another study found that the lower airways of children with severe asthma were enriched with *Bacteroides*, *Faecalibacterium*, and *Roseburia*, compared to children without asthma.⁴³ This group had microbiota enriched for *Proteus* and *Capnocytophaga*, although this study also had a small number of samples.⁴³ Only 1 study compared patients with mild-to-moderate asthma with patients with severe asthma across different age groups, and it found no differences in oropharyngeal microbial communities.⁴⁰ As suggested previously, the differences in composition of the oropharyngeal microbiome may be less explicit in the context of asthma than in other respiratory niches. Currently, no study has investigated the associations between microbiota composition in other respiratory niches and levels of asthma severity; therefore, no definitive conclusions regarding the link between asthma severity and composition of the respiratory microbiota can be made. Studies characterizing the nasopharynx and the lower airways may be needed to determine the relationship between the respiratory microbiome and asthma severity.

The respiratory microbiota and asthma exacerbations

Asthma exacerbations, defined as (sub)acute worsening of symptoms marked by a decrease in lung function from baseline, may interrupt periods of stable disease.¹ Cross-sectional studies have identified a clear association of nasopharyngeal and lower airway microbial communities in children with asthma exacerbations versus in those with stable asthma and healthy controls (Table II).^{32,34} Specifically, children experiencing asthma exacerbations had higher nasopharyngeal *Staphylococcus* and *Streptococcus* levels.³² In contrast, again no differences in oropharyngeal microbiome were described in a study including both children with wheeze and asthma exacerbations,⁴⁴ which may potentially also be explained by the heterogeneous aspect of the patients studied (ie, their broad age range).

Interestingly, the nasal microbiota at times of asthma control (ie, in asthma patients without asthma symptoms) has been correlated with subsequent symptom progression (Table II and Fig 3).^{14,46} Children with mild-to-moderate asthma and nasal

TABLE II. The respiratory microbiota and disease trajectory

Study and niche	No. and type of participants	Definition of asthma outcome	Main findings of interest
Wheeze			
Aydin et al ³² NP	n = 146; children with asthma (n = 46), children with acute exacerbated wheeze (n = 61), and healthy controls (n = 39)	Acute wheeze (at age 3 mo-6 y): 2 episodes of obstructive bronchitis and presentation to the hospital with acute wheeze at study enrollment Asthma (at age 6-17 y): presentation to the hospital with acute asthma at study enrollment Atopic asthma/wheeze: clinical and laboratory-confirmed Nonatopic asthma/wheeze: no clinical or laboratory signs of atopy	Acute wheeze → ↑ <i>M catarrhalis</i> ,* <i>H (para)influenzae</i> ,* <i>S pneumoniae</i> (culture),* ↓ <i>S aureus</i> (culture),* ↑ <i>Proteobacteria</i> ,* <i>Moraxella</i> ,* <i>Haemophilus</i> * (16S) Asthma exacerbation → ↑ <i>Firmicutes</i> ,* <i>Staphylococcus</i> ,* and <i>Streptococcus</i> * (16S) Atopic vs nonatopic wheeze → ↓ (bacterial) diversity Atopic vs nonatopic asthma → ↑ (bacterial) diversity
Tang et al ²¹ NP	n = 289; children had parental history of asthma; followed to age 18 y	Wheezing illness: ≥1 of the following: (1) physician-diagnosed wheeze; (2) prescribed SABA, LABA, and/or long-term controller medication; and (3) diagnosed bronchiolitis, wheezing illness, reactive airway disease, asthma, or asthma exacerbation	Wheeze → ↓ <i>Dolosigranulum</i> † ↑ <i>Streptococcus</i> †
Song et al ³³ NP	n = 70; children with recurrent wheeze (n = 16), inpatient controls (n = 18), and healthy controls (n = 36); aged 2-5 y	Recurrent wheeze: children hospitalized with recurrent wheeze Inpatient controls: children hospitalized for URTI but no history of wheeze/asthma	Recurrent wheeze (vs healthy controls) → ↓ α-diversity and <i>D pigrum</i> * ↑ <i>Proteobacteria</i> *
Chiu et al ³⁴ OP	n = 55; children with asthma (n = 27) and healthy controls (n = 28); aged 3-5 y	Asthma: recurrent wheeze in past 12 mo or current use of asthma medication	Asthma → ↓ <i>Eubacterium sulci</i> ,* <i>Leptotrichia wadei</i> ,* and <i>Prevotella spp</i> * ↑ <i>Neisseria elongata</i> *
Bisgaard et al ³⁵ HP	n = 411; birth cohort (mothers had asthma); followed to age 3 y	Wheezy episodes: parent-reported wheeze for 3 d Objective wheeze: physician-diagnosed Controls: no wheeze Viral infection: RV, RSV, coronavirus, parainfluenza virus, human metapneumovirus, adenovirus, or bocavirus detected during a wheezy episode	(Objective) wheezy episode → ↑ viral infections, <i>H influenzae</i> ,* and <i>M catarrhalis</i> *
Thorsen et al ³⁶ HP	n = 68; episodes of recurrent asthma-like symptoms (n = 139); aged 12-36 mo	Recurrent asthma-like symptoms: parents reported the following: (1) 5 episodes lasting ≥3 d within 6 mo or (2) 4 wk of continuous symptoms or (3) any acute severe episode necessitating hospitalization or (4) treatment with an OCS Primary end point: episode duration, d	Shorter episode-duration → ↑ <i>Moraxella</i> * Longer episode duration → ↑ α-diversity, <i>Neisseria</i> ,†,* <i>Haemophilus</i> ,† <i>Streptococcus</i> ,† <i>Veillonella</i> ,* <i>Prevotella</i> *
Asthma			
Chun et al ³⁷ NC	n = 54; children with asthma (n = 27, median age 11 y [IQR = 8]) and healthy children (n = 27, median age 13 y [IQR = 6])	Severe persistent asthma: Major criteria (≥1 of the following): (1) a high-dose ICS or (2) OCS for ≥50% of the year, and minor criteria (≥2 of the following): (1) additional medication for asthma control, (2) a SABA for ≥5 d per wk, (3) FEV ₁ value < 80% of predicted, (4) ≥1 urgent care visit in within 1 y, (5) ≥3 OCS courses within 1 year, (6) an increase in symptoms after reduction of corticosteroids, and (7) a near-fatal asthma event in the patient's history	Asthma → ↑ <i>Streptococcus</i> *

(Continued)

TABLE II. (Continued)

Study and niche	No. and type of participants	Definition of asthma outcome	Main findings of interest
Depner et al ¹² NC and OP	n = 327; Cross-sectional; children with asthma (n = 125) and healthy controls (n = 202); aged 6-12 y	Asthma: (1) ≥ 2 parent-reported wheeze episodes in 12 mo or less or (2) a positive answer to the question, "Did your child ever use an asthma spray?" or (3) ≥ 1 physician diagnosis of asthma or wheezy bronchiolitis	Asthma (nonfarm children) \rightarrow \downarrow Nasal α - and β -diversity \uparrow Nasal <i>Proteobacteria</i> ,* <i>Moraxella</i> * No differences in OP microbiota
Boutin et al ³⁸ OP	n = 146; cross-sectional; children with asthma (n = 27), children with CF (n = 57), and healthy controls (n = 62); aged 6-12 y	Asthma: (1) ≥ 2 parent-reported wheeze episodes in ≤ 12 mo or (2) a positive answer to the question, "Did your child ever use an asthma spray?" or (3) ≥ 1 physician diagnosis of asthma or wheezy bronchiolitis	Asthma \rightarrow \uparrow <i>Haemophilus</i> *
Espuela-Ortiz et al ³⁹ OP	N = 114; case patients (n = 57) and controls (n = 57); aged 8-21 y	Case patients: physician-diagnosed asthma and active symptoms for ≤ 2 y Controls: patients without any allergy or asthma; no previous wheezing symptoms	Controls \rightarrow \uparrow <i>Streptococcus</i> * Case patients \rightarrow \uparrow α -diversity and <i>Veillonella</i> *
Dzidic et al ²⁹ OP	N = 80; RCT for <i>Lactobacillus reuteri</i> ; children with allergy (n = 47), children with asthma (n = 20), and healthy controls (n = 33); followed from age 3 mo to 7 y	Asthma: ≥ 1 of the following: (1) physician diagnosis and asthma symptoms and/or medication in less than 12 mo or (2) wheeze or nocturnal cough and positive reversibility test result with spirometry at age 7 y	Asthma \rightarrow \downarrow Shannon diversity \downarrow <i>Lactobacillus crispatus</i> * and <i>Lactobacillus gasseri</i> * \downarrow <i>Fusobacterium</i> *, <i>Capnocytophaga</i> *, <i>Lactobacillus</i> *,* and <i>Streptococcus</i> * at age 7 y
Thorsen et al ⁴⁰ OP	n = 241; children with severe asthma (n = 89), children with mild-to-moderate asthma (n = 39), children with severe wheeze (n = 65), and children with mild-to-moderate wheeze (n = 51)	Severe asthma at age 6-17 y: poorly controlled asthma, use of a high-dose ICS and ≥ 2 other control medications Mild-to-moderate asthma at age 6-17 y: (partially) controlled asthma, use of a low-dose ICS and ≤ 1 additional control medication Severe wheeze at age 1-5y: persistent symptoms and frequent exacerbations with use of a high-dose ICS and LTRA Mild-to-moderate wheeze at age 1 to 5 y: (partially) controlled symptoms with no treatment or low-dose ICS and/or LTRA All children: unaltered medication for ≥ 4 wk and no clinical asthma exacerbation (ie, a high-dose OCS for ≥ 3 d or a double OCS dose in cases of maintenance OCS)	No difference in OP microbiota
Bar et al ⁴¹ OP and lower airways†	n = 38; healthy controls (n = 19) and children with asthma (n = 19); aged 6-17 y	Asthma: allergologist-diagnosed "well-controlled" asthma	Asthma \rightarrow higher α -diversity and different overall bacterial community composition No differences in OP microbiota
Al Bataineh et al ⁴² Lower airways§	n = 40; patients with adult asthma (n = 10) and healthy adults (n = 10), as well as patients with pediatric asthma (n = 11; mean age = 67 y) and healthy children (n = 9; mean age = 8 y)	Asthma: physician-diagnosed asthma	Pediatric asthma \rightarrow \downarrow α -diversity, \uparrow <i>Streptococcus</i> *,* and <i>Moraxella</i> *
Hilty et al ¹³ Lower airways	n = 44; adults with asthma (n = 11), adults with COPD (n = 5); healthy controls (n = 8); and children with difficult asthma (n = 13; median age 11.8 y); healthy controls (n = 7; median age 11.3 y)	Pediatric difficult asthma: use of a rescue bronchodilator for ≥ 3 d per wk despite use of a high-dose ICS and LABA and/or OCS	Pediatric difficult asthma \rightarrow \downarrow <i>Bacteroidetes</i> ,† <i>Prevotella</i> ,† \uparrow <i>Proteobacteria</i> ,† <i>Haemophilus</i> ,† and <i>Staphylococcus</i> †

(Continued)

TABLE II. (Continued)

Study and niche	No. and type of participants	Definition of asthma outcome	Main findings of interest
Goldman et al ⁴³ Lower airways	n = 31; children with severe asthma (n = 15; mean age 11.1 years), children without asthma (either normal or structural lesions on bronchoscopy (n = 11; mean age 5.2 years), children with CF (n = 5; mean age 14.4 years)	Severe asthma: ≥1: (1) a high-dose ICS or (2) OCS for ≥50% of the year, and ≥2: (1) additional control medication for asthma control or (2) use of a SABA for ≥5 d per wk or (3) and FEV ₁ value < 80% of predicted or (4) ≥1 urgent care visit in the past year or (5) ≥3 OCS courses in the past year or (6) an increase in symptoms after reduction of corticosteroids or (7) a near-fatal asthma event (requiring intubation) in the patient's history	Asthma → ↑ <i>Bacteroides</i> ,* <i>Faecalibacterium</i> ,* <i>Roseburia</i> * Nonasthma → ↑ <i>Proteus</i> ,* <i>Capnocytophaga</i> *
Exacerbation cross-sectional			
Cuthbertson et al ⁴⁴ OP	n = 184; children with acute wheezing (n = 109; median age 3.83); nonwheezing controls (n = 75); aged 0-16 y	Case patients: included after presentation with acute wheezing illness	No difference in OP microbiota
Kim et al ⁴⁵ Lower airways§	n = 95; children with asthma exacerbation (n = 22; mean age 9 y); children with stable asthma (n = 67; mean age 8 y); healthy controls (n = 6; mean age 13.2 y)	Asthma exacerbation: use of systematic corticosteroids or hospitalization for asthma symptoms Stable asthma: >4 wk without the following: (1) systemic corticosteroid or (2) increased use of and ICS, or (3) rescue treatment for >3× per wk or (4) clinical indication for change in medication Healthy controls: no physician diagnosis of asthma and normal lung function	Exacerbated vs stable asthma → ↓ <i>Saccharimonas</i> , <i>Rothia</i> , <i>Gemella</i> , <i>Bulleidia</i> , and <i>Eubacterium g10</i> ,* ↑ <i>Capnocytophaga</i> * No differences in α-diversity Different overall bacterial community composition
Exacerbation longitudinal			
Zhou et al ¹⁴ NC	n = 214; asthmatic children followed for 48 wk; aged 5-11 y	Stable asthma: physician-diagnosed mild-to-moderate persistent asthma and a daily low-dose ICS and ≥1 asthma exacerbation with use of systemic corticosteroids in the previous year Early loss of asthma control: (1) 2 doses of rescue albuterol in 6 h, or (2) 3 doses of rescue albuterol in 24 h or (3) 1 asthma-related wake-up treated with albuterol Exacerbation: severe asthma exacerbation treated with an OCS	Stable asthma → ↑ <i>Corynebacterium</i> † and <i>Dolosigranulum</i> † → lower risk of early loss of asthma control Early loss of asthma control → switch from <i>Corynebacterium</i> † and <i>Dolosigranulum</i> † to <i>Moraxella</i> † → higher risk of severe asthma exacerbation ↑ α-diversity during early loss of asthma control ↑ <i>Corynebacterium</i> * during early loss of asthma control → lower risk of severe asthma exacerbation
McCauley et al ⁴⁶ NC	n = 181; children with exacerbation-prone asthma during period of respiratory health; aged 6-17 y	Asthma: physician-diagnosed asthma and ≥2 exacerbations in the previous year, nonsmokers with eosinophil counts of >150 cells/mm ³ and required ICS maintenance treatment Exacerbation: systemic corticosteroids or hospitalization	↑ <i>Moraxella</i> * and <i>Haemophilus</i> * → RTI in the fall and subsequent exacerbation ↑ <i>Veillonella</i> , <i>Streptococcus</i> , <i>Neisseria</i> , and <i>Haemophilus</i> network → higher risk of exacerbation ↓ <i>Staphylococcus</i> network → higher risk of exacerbation
McCauley et al ¹⁵ NC	n = 413; asthmatic children participating in omalizumab trial; aged 6-17 y	Exacerbation: systemic corticosteroids or hospitalization	↓ <i>Corynebacterium</i> * and <i>Acinetobacter</i> * at baseline → higher risk of exacerbation ↑ <i>Moraxella</i> † at baseline → higher risk of exacerbation and eosinophil activation ↑ <i>Haemophilus</i> ,† <i>Alloiococcus</i> ,† <i>Corynebacterium</i> ,† or <i>Staphylococcus</i> † → lower risk of exacerbations
Liu et al ⁴⁷ NC	n = 56; asthmatic children; aged 3-17 y	Exacerbation: emergency or outpatient clinic visit for (sub)acute worsening of symptoms, need for treatment, and no fever Recovery: physician-diagnosed asthma for ±2 wk after exacerbation at a routine, nonurgent, asthma FU visit	Recovery to exacerbation: ↓ <i>Moraxella</i> * ↑ <i>Staphylococcus</i> *

(Continued)

TABLE II. (Continued)

Study and niche	No. and type of participants	Definition of asthma outcome	Main findings of interest
Hou et al ⁴⁸ NP	n = 42; children with asthma (n = 33) and healthy controls (n = 9); aged 6-17 y	Asthma: physician-diagnosed asthma with ≥ 1 asthma exacerbation at <12 mo Exacerbation: (1) ≥ 3 doses of a daily SABA for ≥ 2 d or (2) an OCS or (3) asthma-related hospitalization, emergency room or (unscheduled) physician visit Stable asthma: no exacerbation during FU	Stable asthma \rightarrow \uparrow <i>Corynebacterium</i> [†] compared with exacerbation Asthma exacerbation \rightarrow \downarrow <i>Dolosigranulum</i> [*] and <i>Corynebacterium</i> [*] \uparrow <i>Moraxella</i> [*]

CF, Cystic fibrosis; FU, follow-up; HP, hypopharynx; IQR, interquartile range; LABA, long-acting β -agonist; LTRA, leukotriene receptor antagonist; NC, nasal cavity; NP, nasopharynx; OP, oropharynx (or oropharyngeal); RCT, randomized controlled trial; SABA, short-acting β -agonist; URTI, upper respiratory tract infection.

*Genus or species (relative) abundance.

[†]Microbiome profile.

[‡]Lower airways sampled through exhaled breath condensates.

[§]Lower airways sampled through induced sputum.

^{||}Lower airways sampled through bronchoalveolar lavage.

microbiota dominated by *Corynebacterium* and *Dolosigranulum* were protected against an early loss of asthma control, defined as limited need for rescue medication with short-acting β -agonists and nighttime symptoms.¹⁴ Additionally, at times of increased asthma symptoms, higher *Corynebacterium* levels were also associated with a lower chance of requiring subsequent OCS treatment.¹⁴ Conversely, a transition from *Dolosigranulum*- and *Corynebacterium*-dominated microbiota to *Moraxella*-dominated profiles at the start of symptom progression was associated with increased risk of development of a consecutive asthma exacerbation,¹⁴ also suggesting a protective role for *Corynebacterium* and *Dolosigranulum* in symptom progression and subsequent exacerbation. Children with *Moraxella*- and *Haemophilus*-dominated nasal microbiota during stable disease had a higher risk of developing infection symptoms with subsequent asthma exacerbations.⁴⁶

In this and other studies, the respiratory microbiota during asthma control was also directly associated with the development of exacerbations.^{15,46,48} The absence of *Corynebacterium*^{15,48} and *Acinetobacter*¹⁵ and microbiota not dominated by *Haemophilus*, *Dolosigranulum*, or *Staphylococcus* during disease control were related with the development of exacerbations.¹⁵ Additionally, a *Moraxella*-dominated nasal microbiota was associated with an increased risk of exacerbation.¹⁵ Another study showed that the microbial network at baseline was associated with a risk of future exacerbation, with networks dominated by *Veillonella*, *Streptococcus*, *Neisseria*, and *Haemophilus* being associated with a higher risk and *Staphylococcus*-dominated networks being associated with a lower risk of future exacerbations.⁴⁶ Together, these studies suggest a potential protective effect of certain microbes, especially *Corynebacterium* and *Staphylococcus*, against future exacerbations.

The dynamics of the nasopharyngeal microbiota further showed a decrease in *Corynebacterium* and *Dolosigranulum* levels and an increase in *Moraxella*-dominated profiles during exacerbation.⁴⁸ Following exacerbation, the microbiota of most children recovered from a *Moraxella*-dominated cluster to a *Corynebacterium* and *Dolosigranulum*-dominated cluster.⁴⁸ Similarly, another study found a decrease in nasal *Moraxella* and an increase in nasal *Staphylococcus* levels between exacerbation and recovery.⁴⁷ In summary, upper airways with a *Corynebacterium* and *Dolosigranulum*-dominated microbiota were often associated with asthma control and fewer exacerbations (Table II and Fig 3).

THE RESPIRATORY MICROBIOME IN RELATION TO UNDERLYING IMMUNE RESPONSE

Early-life colonization and potential mechanisms that affect asthma development

We have described how early-life microbial colonization is associated with subsequent development of asthma (Table I and Fig 1). An important source of the early-life microbiota is the child's mother. A recent study showed that 58.5% of the infant microbiota originates from the mother.⁴⁹ Another study suggested that besides being an important source of the infant microbiome, mothers also transfer DNA methylation signatures to the newborn via cord blood mononuclear cells, which could subsequently affect the composition of the nasopharyngeal microbiota.⁵⁰ Specifically, mothers (particularly nonasthmatic mothers) with altered immunity during pregnancy (defined as a decreased IFN- γ /IL-13 ratio in the third trimester), gave birth to children with hypermethylation of genes associated with microbial immune responses, including genes involved in a TGF- β pathway, a gene that dampens cytokine response to gram-negative bacteria, and a chemokine receptor implicated in leukocyte migration. In addition, these children showed increased risk of childhood asthma and demonstrated *Haemophilus*-dominated nasopharyngeal microbiota in their first year of life, with subsequent transition to *Moraxella*-dominated microbiota profiles at around age 3 years.⁵⁰ Although these microbial signals were not directly related to asthma development in this study,⁵⁰ they have (as described earlier in this article) been associated with later asthma development in other works.^{9,18,22} This suggests that an altered early-life microbiota can be a consequence of both inherited microbes and inherited epigenetic traits associated with asthma.

In addition, altered inherited or acquired early-life microbiota could also contribute to the development of asthma by influencing normal immune development. For example, studies in germ-free mice have shown aberrant spleen and lymphoid node formation, which was associated with reduced or absent induction of regulatory T (Treg) cells, T_H17 cell presence, and an imbalance in T_H2 and T_H1 cells.^{51,52} Treg cells restrain T_H2 and T_H17 cell immune responses, and their levels are reduced in asthma. As mentioned earlier, *Lactobacillus* has a beneficial association with later asthma development (Table I and Fig 1) and has also been found to induce Treg cells that can protect against allergic airway responses in mice.⁵³ This illustrates a potential

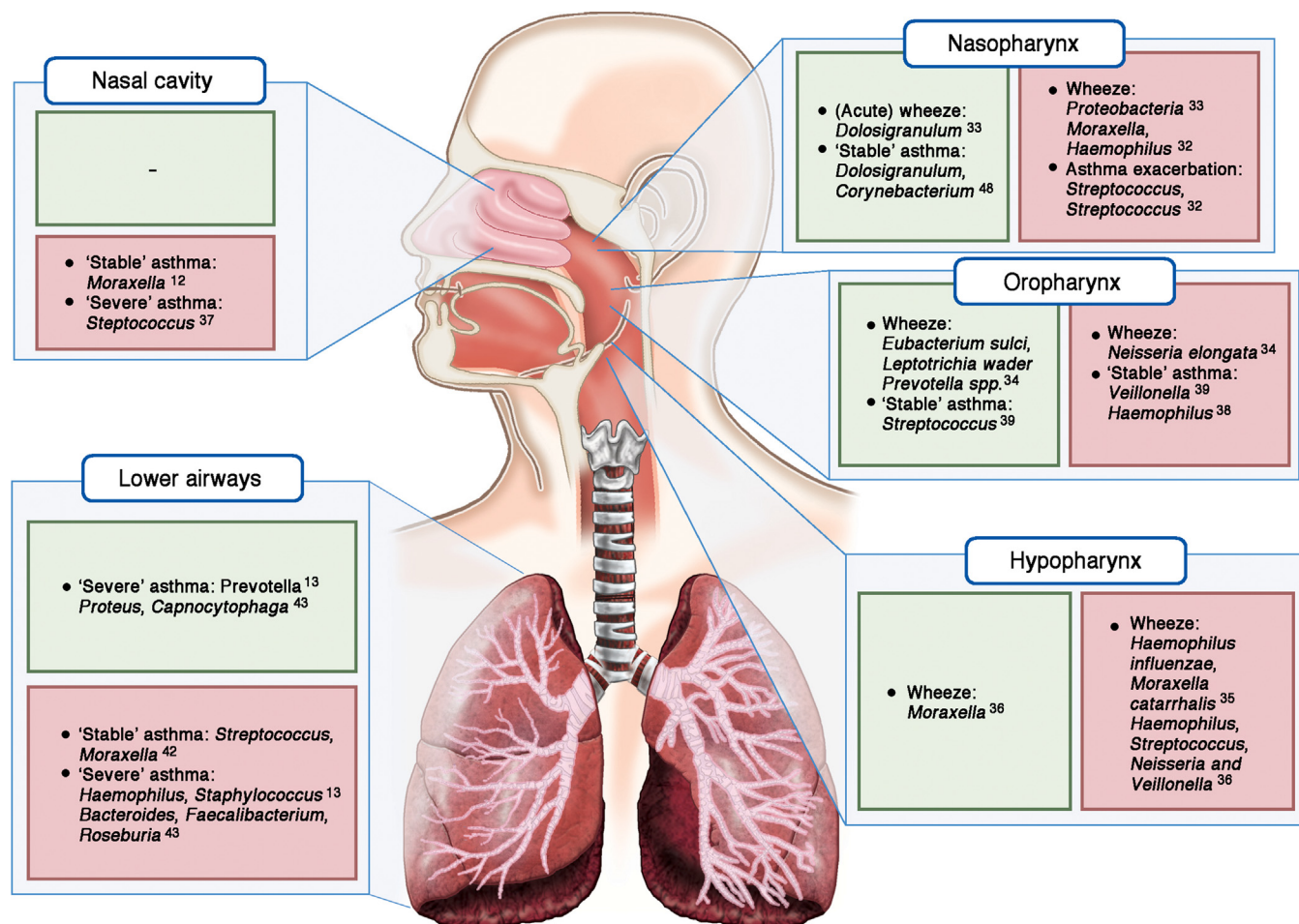


FIG 2. Respiratory microbiota signals related to existing disease in children. Microbiota represented in green boxes are associated with healthy controls or fewer asthma and/or wheeze symptoms, whereas microbiota in the red boxes are associated with having asthma or wheeze symptoms. Results are stratified by respiratory niche and asthma (in patients aged ≥ 5 years) or wheeze (in patients aged < 5 years).

mechanism through which early-life microbiota may protect against asthma development. Additionally, findings from murine models indicate that strongly reduced exposure to bacteria (including germ-free mice, germ-free living conditions, and administration of antibiotics) is associated with higher levels of IgE, airway eosinophils, and T_H2 cytokines (ie, IL-4, IL-5, IL-13),⁵⁴⁻⁵⁷ as well as with increased airway hyperresponsiveness and T_H17 cell levels,⁵⁸ suggesting that alterations in the early-life microbiome may contribute to asthma development.

The respiratory microbiota in relation to high T_H2 and low T_H2 cell endotypes

Asthma is a heterogeneous disease and is often stratified into different phenotypes based on clinical characteristics. A common distinction in clinical phenotypes is made between atopic and nonatopic wheeze or asthma. Alternatively, asthma can be classified by underlying pathophysiologic mechanisms (endotype), with the literature currently distinguishing T_H2 cell-high and T_H2 cell-low asthma endotypes.⁵⁹

The T_H2 cell-high endotype is correlated with an atopic phenotype and characterized by T_H2 cell and type 2 innate lymphoid

cell mediated inflammation (IL-4, IL-5, and IL-13) and high eosinophil levels. The presence and abundance of *Moraxella* in the respiratory tract seems to be associated with both the clinical atopic wheeze or asthma phenotype,^{60,61} as well as with cytokines and other immunologic features related to a T_H2 cell-high endotype.^{9,15,24,32} Specifically, high nasal *Moraxella* levels⁶⁰ and lower airway cultures that are positive for *M catarrhalis*⁶¹ were associated with clinical atopic clusters in children with physician-diagnosed asthma or recurrent wheeze, respectively. Additionally, sensitized children were more likely to have a RV infection and positive lower airway cultures for *M catarrhalis*.⁶¹ Conversely, nonatopic clusters demonstrated low nasal *Moraxella* and *Dolosigranulum* levels and high *Corynebacterium* and *Prevotella* levels,⁶⁰ as well as lower airway cultures positive for *H influenzae*, *S aureus*, and *S pneumoniae*.⁶¹ One small study, however, did not observe a difference in the dominant lower airway microbiota between atopic and nonatopic wheeze.⁶² Studies have also linked *Moraxella* to immunologic features of the T_H2 cell-high endotype. For example, early-life colonization with *S pneumoniae*, *M catarrhalis*, or *H influenzae* has been related to elevated total IgE levels later in childhood.⁹ In addition, children who are hospitalized with bronchiolitis in their first year of life and

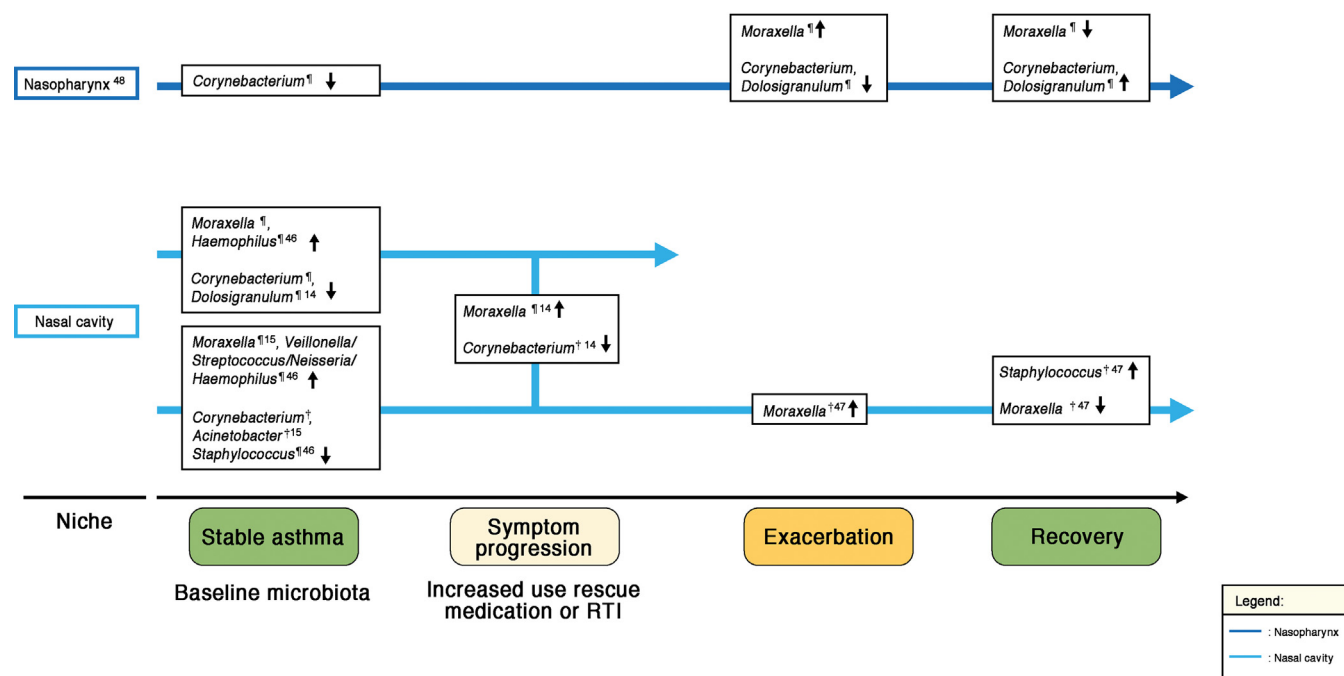


FIG 3. Respiratory microbiota before and after asthma exacerbations. Upward arrows in boxes indicate positive associations between respiratory microbiota and subsequent development of a loss of asthma control, exacerbations, and recovery to a baseline state, whereas downward arrows show negative associations. Lines are colored by respiratory niche. ¶Microbiome profile. †Genus or species (relative) abundance.

belong to a cluster with high levels of T_H2 cytokines (IL-4, IL-5, and IL-13), blood eosinophils, and IgE sensitization had *Moraxella*-dominated nasopharyngeal microbiota and the highest risk of developing recurrent wheeze and asthma.²⁴ This bronchiolitis cluster also showed high levels of epithelial-derived cytokines (IL-25, IL-33, and thymic stromal lymphopoietin [TSLP]),²⁴ which have been implicated in the T_H2 cell–high asthma endotype.⁵⁹ Moreover, nasal epithelium cultures exposed to *M catarrhalis* showed a disruption of epithelial cell integrity, cytokine release, and cohort-specific proteomic differences.³² Similarly, *M catarrhalis* induced greater epithelial damage and release of inflammatory cytokines (IL-33 and IL-8) than did *Staphylococcus epidermidis*, *S aureus*, and *Corynebacterium propinquum*.¹⁵ Lastly, oropharyngeal *Streptococcus intermedius* has also been associated with total and mite-specific IgE levels in children with mite-sensitized asthma.³⁴ Taken together, these findings imply a relation between *Moraxella* and the T_H2 cell–high asthma endotype.

In contrast, the T_H2 cell–low asthma endotype is associated with T_H17 or T_H1 cell mediated inflammation and is characterized by higher neutrophil levels and the production of IL-17 and IFN- γ .⁵⁹ Neutrophilic endotypes are often associated with a severe asthma phenotype.¹ Hospitalized children with bronchiolitis who belong to a cluster with low levels of T_H2 cytokines and RV type A infection had *Haemophilus*-dominated microbiota.²⁴ *H influenzae* has also been associated with upregulation of T_H17 cell genes in children who showed an increased risk of developing asthma after bronchiolitis hospitalization.²⁶ In line with these findings, *Haemophilus* predominance has also been associated with higher CXCL8 (previously IL-8) levels,³⁰ a cytokine that plays a role in neutrophil recruitment. One study in mice

found that *H influenzae* infection induces pulmonary IL-17 expression,⁶³ suggesting that *H influenzae* could incite cytokines that are associated with a T_H2 cell–low endotype. Neutrophil counts were also a strong predictor of microbiome composition in adults with severe asthma, with higher *Haemophilus* levels and lower *Gemella* and *Streptococcus* levels being associated with neutrophilic asthma.⁶⁴ *Moraxella*-dominated microbiota, like *Haemophilus*-dominated microbiota, were also associated with T_H2 cell–low asthma endotypes, higher levels of neutrophils in bronchoalveolar lavage fluid,⁶² and IL-6 and IL-10 levels.³⁰ In summary, respiratory microbiota dominated by *Moraxella* were predominantly associated with the T_H2 cell–high endotype, whereas those with *Haemophilus* were associated with T_H2 cell–low endotypes. The question remains as to whether these microbial signatures contribute directly to asthma etiology and symptoms, reflect underlying immune alterations, or are merely innocent bystanders.

DISCUSSION AND CONCLUSION

We here have described the pediatric respiratory microbiota in relation to asthma development, disease trajectory, and underlying immune response. Despite the different phases in the disease etiology, the heterogeneity of asthma phenotypes and endotypes and the different respiratory niches studied, we find clear microbial signatures that are associated with asthma development and disease course. Notably, multiple studies have linked levels of early-life *Streptococcus* (*S pneumoniae*), *Moraxella* (*M catarrhalis*), and *Haemophilus* (*H influenzae*) with subsequent asthma development. These same microbes were also associated with wheeze, asthma, symptom progression, and exacerbations. These

bacteria, particularly *M catarrhalis* and *H influenzae*, are considered potential pathogens and thrive in inflammatory climates.⁶⁵ Conversely, lactate acid-producing bacteria (*Lactobacillus* and *Dolosigranulum*) and *Corynebacterium* were frequently associated with healthy children, stable asthma, and/or lower risk of exacerbation. Lactic acid is considered a beneficial product of the host microbiota, as it modulates inflammatory cytokines⁶⁶ and stimulates the short-chain fatty acid production of butyrate.⁶⁷ Early antibiotic treatment in mice reduced butyrate and increased IgE levels and asthma susceptibility, whereas administering short-chain fatty acids (including butyrate) restored IgE levels and asthma susceptibility.⁶⁸ This provides a potential mechanism by which commensal microbiota and their products can contribute to the disease course of asthma. In addition, co-occurrence of *D pigrum* and *Corynebacterium* has been associated with control of respiratory viruses^{10,31} and inhibition of *in vitro* *S pneumoniae* growth,⁶⁹ suggesting that together these taxa could decrease exposure to viral triggers of asthma and asthma-associated bacteria. These examples underline a potential biologic mechanism explaining the association of these bacteria with health.

These microbial signals could potentially serve several clinical applications. First, they can be used as biomarkers for diagnostic and management strategies. For example, oropharyngeal microbial profiles have been associated with specific clinical traits, including comorbidities (atopic dermatitis and allergic sensitization) and future exacerbations,⁷⁰ offering a noninvasive tool to support management of childhood wheeze and asthma.

Second, the described microbial signals may provide a therapeutic target by itself⁷¹ (for example, by using antibiotics). It has already been proved that azithromycin reduces exacerbations in both asthma⁷² and chronic obstructive pulmonary disease (COPD).⁷³ Erythromycin therapy has also been proved to reduce the number of exacerbations in a different patient group (eg, patients with bronchiectasis).⁷⁴ It has been suggested that these effects are mostly a consequence of the immunomodulatory properties of macrolides, but there is also evidence for their direct antimicrobial effect. Specifically, 1 of the aforementioned studies showed that the efficacy depended on the dominant bacterial taxon in the respective patients' microbiome.⁷⁴ That macrolides may act through microbial effects is further supported by the finding that azithromycin reduces the duration of recurrent asthma-like symptoms, especially in children who have high bacterial diversity and specific bacteria in the hypopharynx.³⁶ Lastly, 1 study showed that azithromycin treatment for COPD increased levels of microbial metabolites (eg, glycolic acid, indole-3-acetate). Subsequently, in *ex vivo* models these metabolites were found to decrease proinflammatory cytokines (ie, TNF- α , IL-12 p40, IL-13, and CXCL-1), whereas azithromycin did not,⁷⁵ which strongly suggests that the anti-inflammatory effects of azithromycin can be mediated by microbial metabolites. Importantly, these results indicate that microbial biomarkers could be used to identify children who may benefit from macrolide or (potentially) other tailored antimicrobial treatments to reduce asthma exacerbations.

Third, the microbes themselves could theoretically be used to reverse microbial dysbiosis and thereby help to prevent disease development and severity. This can be done indirectly via immunomodulatory effects or directly through competition with proinflammatory microorganisms and restoration of a healthy microbiome. An example of the first approach is the

administration of bacterial lysates (such as MV130 and OM85), which include lysed respiratory pathogens (such as *H influenzae*, *M catarrhalis*, and *S pneumoniae*). Such lysates have already been demonstrated to modulate the immune response and consequently protect against viral respiratory infections⁷⁶ and development of allergic asthma⁷⁷ in mouse models. Clinical trials in children have also shown that these bacterial lysates reduce the number of recurrent respiratory infections,⁷⁸ the number and duration of wheeze attacks,⁷⁹⁻⁸¹ and the number of asthma exacerbations.⁸²⁻⁸⁴ An example of the second approach may be administration of novel live biotherapeutic products, including commensals such as *Lactobacillus*, *D pigrum*, and *Corynebacterium*. Murine infection models showed that administering specific strains of these bacteria reduced inflammation and increased protection against RSV with or without *S pneumoniae*,⁸⁵ both of which are also associated with wheeze and asthma development and severity. Additionally, a first human trial using a live lactobacilli throat spray in healthy adults demonstrated immunostimulatory and antiviral effects.⁸⁶ Administering local biotherapeutics may therefore be the next step in restoring a healthy microbiome and thereby preventing wheeze and asthma development or treating disease severity. Because of anatomic differences in the respiratory tract between infants and older children, the seeding of the lower airways likely originates from different upper airway niches depending on anatomic age. The lungs of infants are likely seeded primarily by the nasal and nasopharyngeal niche, whereas like the lungs of adults, the lungs of older children may be influenced more by oropharyngeal microbiota.⁶ This could potentially explain some of the variation in findings from respiratory microbiome studies across the age span. Together with the variable physiologic conditions throughout the respiratory tract, this underlines the benefit of incorporating multiple respiratory niches into respiratory microbiome studies.

Although the respiratory microbiome is an important entity to study in relation to asthma, it should be noted that the gut microbiome has also been implicated in this disease, via the so-called gut-lung axis.^{87,88} Furthermore, there is increased interest in the interplay between the respiratory and gut microbiomes in general and in relation to asthma.⁸⁹⁻⁹¹ These topics fall outside the scope of the current review article, but other reviews provide more information on these subjects, including the reviews by Huang and Boushey⁹² and Abdel-Aziz et al.⁹³ Recent evidence also expands on the role of viruses^{94,95} and fungi,^{43,96-98} which together with bacteria form an intrinsic part of the microbiome, and as such, they could directly or through interplay influence asthma outcomes.

In conclusion, we found aberrant microbial development patterns in early life that are associated with development of infections and consecutive wheeze and asthma. Notably, the microbes associated with this chain of events (both proinflammatory bacteria such as *M catarrhalis*, *S pneumoniae*, and *H influenzae* and health-associated bacteria such as *D pigrum*, *Corynebacterium*, and *Lactobacillus*) are also linked with development of or protection against other respiratory diseases, such as acute and chronic respiratory tract infections^{31,99} and COPD.¹⁰⁰ Consequentially, these entities seem interrelated with development of the respiratory microbiome. The development of the respiratory microbiome is significantly influenced by early-life factors such as mode of birth, breast-feeding, and maternal and infant antibiotic treatments. It therefore seems plausible that these

early-life factors could thus in part induce or mediate long-term respiratory health.

Future research should focus on early-life events that may be connected via respiratory microbiota with development and severity of acute infections as well on as consecutive chronic respiratory diseases such as asthma and COPD, as this may be an important modifiable trait that could improve long-term respiratory health.

DISCLOSURE STATEMENT

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