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Developmental patterns in the nasopharyngeal microbiome during infancy are associated with asthma risk

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Background: Studies indicate that the nasal microbiome may correlate strongly with the presence or future risk of childhood asthma.

Objectives: In this study, we tested whether developmental trajectories of the nasopharyngeal microbiome in early life and the composition of the microbiome during illnesses were related to risk of childhood asthma.

Methods: Children participating in the Childhood Origins of Asthma study (N = 285) provided nasopharyngeal mucus samples in the first 2 years of life, during routine healthy study visits (at 2, 4, 6, 9, 12, 18, and 24 months of age), and during episodes of respiratory illnesses, all of which were analyzed for respiratory viruses and bacteria. We identified developmental trajectories of early-life microbiome composition, as well as predominant bacteria during respiratory illnesses, and we correlated these with presence of asthma at 6, 8, 11, 13, and 18 years of age.

Results: Of the 4 microbiome trajectories identified, a Staphylococcus-dominant microbiome in the first 6 months of life was associated with increased risk of recurrent wheezing by age 3 years and asthma that persisted throughout childhood. In addition, this trajectory was associated with the early onset of allergic sensitization. During wheezing illnesses, detection of rhinoviruses and predominance of Moraxella were associated with asthma that persisted throughout later childhood.

Conclusion: In infancy, the developmental composition of the microbiome during healthy periods and the predominant microbes during acute wheezing illnesses are both associated with the subsequent risk of developing persistent childhood asthma. (J Allergy Clin Immunol 2020;146:1379-1389)

Key words: Microbiome, children, asthma, development, birth cohort

Asthma is a chronic inflammatory disease that affects 6 million children in the United States alone. Although childhood asthma can be treated, the lack of a cure underscores the need to understand its early-life developmental origins. Most cases of persistent childhood asthma begin with acute infectious wheezing illnesses in infancy. Although these illnesses are initiated by respiratory viruses, there is strong evidence that bacterial pathogens also contribute, and both types of microorganisms have also been related to the subsequent risk of developing asthma. Wheezing illnesses caused by respiratory syncytial virus (RSV) and rhinovirus (RV) are associated with asthma, especially in children who develop early allergic sensitization. In addition, detection of specific bacteria by culture (Streptococcus pneumoniae, Moraxella catarrhalis, or Haemophilus influenzae) or 16S sequencing (eg, Prevotella, Veillonella) in oral or nasopharyngeal aspirates of babies have been related to asthma in early childhood. In an Australian birth cohort (Childhood Asthma Study) using bacterial metagenomics based on 16S rRNA, predominance of S pneumoniae, M catarrhalis, or H influenzae was found to interact with early allergic sensitization to increase the risk of later asthma. Others have found coassociation between eosinophil counts, severe RV bronchiolitis, and a Haemophilus- or Moraxella-dominated profile of nasopharyngeal microbiota in infants. These studies suggest that infection by viral and bacterial pathogens promotes acute wheezing illnesses and increases the risk of asthma while possibly interacting with other host factors such as allergy. There is also considerable interest in determining whether the dynamic transformation of the airway microbiome—its developmental pattern—is associated with acute or chronic respiratory illness. The composition of the airway microbiome...
typically undergoes marked changes in the first postnatal weeks and months, and this process can be influenced by mode of delivery,\textsuperscript{15,16} viral illnesses\textsuperscript{17,18} and exposure to other children.\textsuperscript{17} Given the likewise rapid maturation of mucosal immunity in early life, host microbiome dynamics during early childhood may affect future health and disease through interactions with immune development.\textsuperscript{8,19}

Collectively, these findings suggest that both the developmental trajectory of the airway microbiome in early life and episodic incursions with viral and bacterial pathogens during respiratory illnesses modify the risk of developing childhood asthma. To further test these hypotheses, we analyzed respiratory bacteria and viruses in nasopharyngeal mucus specimens collected from children enrolled in the Childhood Origins of Asthma (COAST) study under 2 sets of conditions: (1) multiple scheduled visits mostly during periods of good health through 24 months of age and (2) acute respiratory illnesses.\textsuperscript{20} We derived developmental trajectories of airway microbiome assembly based on the routine samples and then tested these trajectories and microbial composition during respiratory illnesses for associations with asthma throughout childhood.

METHODS
Study design
Participants of the COAST birth cohort study (initial N = 289)\textsuperscript{20} were recruited in Madison, Wisconsin, and surrounding areas from November 1998 to May 2000. The study was approved by the University of Wisconsin-Madison Human Subjects Committee. All families provided informed consent before enrollment, and children provided assent when they reached 7 years of age. All recruited children had at least 1 parent with an allergic disease or asthma. Routine scheduled nasopharyngeal sampling was performed at the time points of 2, 4, 6, 9, 12, 18, and 24 months of age. Most routine samples were collected from children during periods of good health, although some coincided with symptoms of mild respiratory illness. From birth until age 3 years, additional samples were collected from children with upper respiratory illness of at least moderate severity, or with any lower respiratory illness, as previously described.\textsuperscript{28,29}

The children had yearly routine visits to the clinic where they underwent procedures including assessment of IgE sensitization to aeroallergens (cat, dog, Dermatophagoides pteronyssinus, Dermatophagoide fariniae, and Alternaria), blood eosinophil counts, lung function, and asthma diagnosis from ages 6 to 18 years.\textsuperscript{22} Information on environmental exposures and allergic conditions was collected. Wheezing illnesses, rhinitis, asthma, and atopic dermatitis latent classes were defined as previously described.\textsuperscript{11,12,23-25}

Detection of viruses and bacteria
We performed 16S rRNA amplicon sequencing of nasopharyngeal samples (swab or aspirate) and negative controls.\textsuperscript{7} Microbiome data were processed by using QIIME2 (version 2017.10/12)\textsuperscript{26} and DADA2\textsuperscript{27} to produce relative abundance data for amplicon sequence variants (ASVs), representing unique 16S rRNA V4 sequences. The nasopharyngeal samples were clustered into microbiome profile groups (MPGs) by using hierarchic clustering methods.\textsuperscript{5,7} Nasal specimens were analyzed for common respiratory viruses as previously described.\textsuperscript{28,29}

Statistical methods
We used the relative abundances of common ASVs to determine clusters of individuals who shared similar patterns (“trajectories”) of changing microbiome during routine visits (with samples from healthy or mildly ill patients). To generate these trajectories, we omitted all samples obtained at 18 months of age because of the high rate of missing samples at this time point. We then performed multiple-factor analysis (using the R package FactoMineR),\textsuperscript{30} followed by k-means clustering.

To estimate a longitudinal asthma phenotype, simple latent class models were fit by using asthma diagnoses at ages 6, 8, 11, 13, and 18 years as variables. Next, conditional variable importance measures from random forest ensembles were used to identify microbial and viral features (MPG wheezing burdens, viral wheezing episodes, and routine visit microbiome trajectory) for additional analysis based on associations with the 4-class asthma phenotype.

To compare MPGs and multiple-factor analysis–k-means trajectories, Fisher exact tests and chi-square tests were used for categoric variables; Kruskal-Wallis, t tests, and ANOVA were used for continuous variables. More complex associations were assessed by using generalized linear models for subject-based analyses, or generalized estimating equations (GEEs), using the R package gee [version 4.13.20]\textsuperscript{31,32} for sample-based analyses with adjustment for sex, age, and season with repeated measures of multiple samples per child subject and unstructured correlation. These analyses were conducted by using R software, version 3.5.0. Post hoc comparisons with false discovery rate correction were conducted where required.

Additional details on study and statistical methods are listed in the Online Data Supplement (in this article’s Online Repository at www.jacionline.org).

Data and material availability
The microbial sequences have been uploaded to GenBank (accession number pending).

RESULTS
Composition of nasopharyngeal microbiome in COAST
A total of 3147 nasal samples were analyzed for bacteria, including 1654 samples collected during routine scheduled visits (at 2, 4, 6, 9, 12, 18, and 24 months of age) and 1493 additional specimens collected during respiratory illnesses. Of these samples, 2922 passed quality controls (1488 routine and 1434 episodic), of which 1059 were routine and from truly healthy patients, whereas 1863 were samples from patients with illness or mild illness that were collected during either routine or episodic visits. There were 414 distinct samples corresponding to wheezing illnesses. The most common ASVs belonged to 6 genera; Dolosigranulum, Corinebacterium, Haemophilus, Staphylococcus, Streptococcus, and Corynebacterium. These ASV sequences most closely matched those of the bacterial species Dolosigranulum pigrum, Corinebacterium pseudoplihetericum, 2 subtypes of H influenzae, M catarrhalis, S pneumoniae, multiple Staphylococcus species (including S aureus and S epidermidis), and Streptococcus mitis, respectively (see Table E1 in this article’s Online Repository at www.jacionline.org).

Clustering into MPGs
Consistent with previous similar studies,\textsuperscript{5,7} each nasopharyngeal sample had a simple structure, being largely dominated

| Abbreviations used |
|--------------------|
| ASV: Amplicon sequence variant |
| COAST: Childhood Origins of Asthma birth cohort study |
| GEE: Generalized estimating equation |
| MPG: Microbiome predominance group |
| RSV: Respiratory syncytial virus |
| RV: Rhinovirus |

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Consistent with previous similar studies, each nasopharyngeal sample had a simple structure, being largely dominated
Streptococcus)—from healthy children), MPGs with acute wheezing illnesses (compared with samples from sick children. In a similar analysis testing for association of mitis showed a significant positive association (2.6 ± 3).

Conversely, MPGs dominated by dodiphtheriticum showed a negative association (P < 10–5). Similar results were attained for any pathogen MPG, odds ratio (OR) = 5.3575. The presence of any pathogen MPG and the presence of virus each remained independently associated with respiratory illnesses, even with adjustment for each other, age, sex, and seasonality (GEE model, for any pathogen MPG, odds ratio = 3.4 and P = 7.3 × 10–8; for any virus, odds ratio = 12 and P < 1 × 10–10).

**Trajectory analysis of the nasopharyngeal microbiome**

Nasopharyngeal samples from routine study visits across the first 2 years of life were analyzed to identify temporal trajectories of microbiome assembly. We identified 4 clusters of children (>50% of reads per sample) by a single ASV. Hierarchic clustering identified 12 MPGs. Each MPG described a pattern with a single dominant ASV and was named according to this dominant taxon. Incidentally, the 12 MPGs corresponded to the most abundant ASVs (Fig 1, A, and for relative abundances for all features, see Fig E1 in this article’s Online Repository at www.jacionline.org).

**MPG association with acute respiratory illness**

Four specific MPGs were significantly overrepresented in the samples from children with respiratory illness compared with samples from healthy children (P < .05 [Fig 1, B and see Table E2, A in this article’s Online Repository at www.jacionline.org]). These MPGs were those of known respiratory pathogens Moraxella.d253 (M catarrhalis), Streptococcus.4060 (S pneumoniae), Haemophilus.1579, and Haemophilus.bc0d (both H influenzae). Conversely, MPGs dominated by Corynebacterium.cb50 (C pseudodiphtheriticum), Dolosigranulum.dd2e (D pigrum), Staphylococcus.29eb (Staphylococcus spp), and Streptococcus.3575 (S mitis) were more common in samples from healthy rather than sick children. In a similar analysis testing for association of MPGs with acute wheezing illnesses (compared with samples from healthy children), Streptococcus.4060 (S pneumoniae) showed a significant positive association (P = .00035) and Dolosigranulum.dd23 (D pigrum) showed a negative association (P = 2.6 × 10–5 [see Table E2, A]). Similar results were attained after adjustment for other asthma-related covariates, including parental allergy, parental asthma, environmental smoke exposure, presence of pets, breast-feeding, and birth by Cesarean (see Table E2, B).

As noted in our previous publications,33,34 the viruses most commonly detected in the specimens were RV, RSV, parainfluenza virus, coronavirus, and metapneumovirus. We observed that pathogen MPGs (Moraxella.d253, Streptococcus.4060, Haemophilus.1579, and Haemophilus.bc0d) and certain respiratory viruses (RSV and influenza) often coexisted in the same sample, especially during illnesses in the winter months (see Figs E2 and E3 in this article’s Online Repository at www.jacionline.org). The distribution of detected MPGs was generally similar across all viruses, whether we examined all samples or only those samples from wheezing illnesses (see Fig E3). During illnesses (n = 1863), pathogen-related MPGs and viruses were most often detected together (n = 1224 [66%]), followed by viruses alone (n = 422 [23%]), pathogen-related MPGs alone (n = 145 [7.8%]), and neither (n = 72 [3.9%]). The presence of any pathogen MPG and the presence of virus each remained independently associated with respiratory illnesses, even with adjustment for each other, age, sex, and seasonality (GEE model, for any pathogen MPG, odds ratio = 3.4 and P = 7.3 × 10–8; for any virus, odds ratio = 12 and P < 1 × 10–10).
distinguished by distinct patterns of microbial composition over
time (Fig 2, A). Each trajectory appeared to be driven by a
different MPG in the first 6 months of life: trajectory A (n =
79) appeared to be driven by Dolosigranulum.dd2e and Coryne-
bacterium.cb50; trajectory B (n = 43) appeared to be driven by
Moraxella.d253; trajectory C (N = 26) appeared to be driven by
Staphylococcus.29eb; and trajectory D (n = 135) appeared to
be driven by Streptococcus.4060 and other streptococci. Because
V3 and V4 primers do not reliably differentiate S. aureus and S.
epidermidis, 20 trajectory C nasal mucus samples obtained at 2
months of age were analyzed by quantitative PCR, revealing a
predominance of S. aureus (see Fig E4 in this article’s Online Re-
pository at www.jacionline.org).

Notably, as the children grew older, the trajectories became
more similar, and by age 2 years, they had converged toward a
generally mixed composition (with many dominated by
Moraxella.d253). At age 2 months, between-trajectory dissimilarity
(Bray-Curtis) was greatest (0.86), whereas the dissimilarity
within the same trajectory was smallest (0.55). These dissimilar-
ities gradually shifted with age, until by age 2 years both be-
 tween-and within-trajectory dissimilarities were roughly equal
(0.71).

During wheezing illnesses, nasal bacteria were typically
dominated by illness-associated taxa (eg, Moraxella.d253, Strep-
tococcus.4060, and Haemophilus taxa) irrespective of trajectory
(Fig 2, B). There were no significant differences in the rate of
detection of specific viruses between any of the 4 trajectories in
routine samples or in wheezing illness samples (see Fig E5 in
this article’s Online Repository at www.jacionline.org).

Demographic characteristics were similar among children in
the 4 trajectories (Table I). There were no significant differences
among the microbiome trajectories in terms of other environ-
mental variables, including mode of delivery, presence of pets
(cat, dog) in the home, number of siblings at time of birth, exclu-
sive breast-feeding during the first 6 months of life, and antibiotic
use in the first year of life (Table I).

Association of microbiome trajectories with early
wheezing illness and later asthma

Trajectory C, dominated by Staphylococcus.29eb, was associ-
ated with the greatest frequency of wheezing illness in the first 3
years of life; however, this association differed by age (Fig 3).
The number of wheezing illnesses per trajectory was most similar
in the first year of life, lowest for trajectories A (Dolosigranulum)
and C (Staphylococcus), and highest for trajectory D (S. mitis).
However, trajectory C was associated with a progressive increase
in wheezing illnesses with time, overtaking the other trajectories
to give the greatest frequency at year 3 (P = 0.0006 for trajectory C).
In addition, trajectory C was also associated with greater frequency of physician-diagnosed asthma in years 6, 13, and 18 compared with the other trajectories (Fig 4, A). Furthermore, we applied a latent class model to asthma diagnoses at age 6, 8, 11, 13, and 18 years to identify 4 longitudinal patterns of asthma (see Fig E6 in this article’s Online Repository at www.jacionline.org): none or intermittent (63% of subjects), persistent (19% of subjects), remitting (10% of subjects), and late-onset (8% of subjects). Compared with the other microbiome trajectories, trajectory C (Staphylococcus.29eb dominance) tended to be positively associated with a persistent asthma phenotype (P = .008 [Fig 4, B]).

We next evaluated microbial predictors of asthma phenotypes in a random forest model that included the routine visit microbiome trajectories together with MPG and virus detection during wheezing illnesses (see Fig E7 in this article’s Online Repository at www.jacionline.org). In the first year of life, microbiome trajectory C along with detection of illness-associated MPGs (Moraxella.d253, Haemophilus.bc0d) were most predictive of asthma class. When the predictors were evaluated over the first 3 years, the microbiome trajectory was no longer among the key predictors of asthma class. Instead, detection of RV during illnesses was an important predictor, and illness-associated MPG Moraxella.d253 remained an important asthma class predictor. These relationships were modified by the age of the child at the time of the wheezing illness (Fig 5). Both RV and Moraxella.d253 wheezing illnesses in the first year of life were modestly associated with the persistent asthma latent class, whereas wheezing illnesses associated with RV or Moraxella.d253 during years 2 and 3 were strongly related to persistent asthma.

### Association of microbiome trajectories with allergy variables

Given the close association between early onset of atopy and persistent asthma, we next tested for associations between microbiome trajectory C and indicators of type II inflammation and allergic outcomes. Trajectory C was associated with a greater frequency of aeroallergen sensitization, especially during early childhood (Fig 6, A). The difference between trajectory C and the others was significant through to age 5 (P < .05 at each age) and also when all years were considered together (trajectory C vs others; P = .05). There were similar nonsignificant trends for associations between trajectory C and increases in both total IgE level and absolute eosinophil counts (Fig 6, B and C). Trajectory C was associated with a nonsignificant trend for increased risk of allergic rhinitis at age 6 years (overall P = .05, trajectory C vs others P = .12), but not with early-onset atopic dermatitis (see Fig E8 in this article’s Online Repository at www.jacionline.org) or lung function (FEV1 or FEV1-to Forced Vital Capacity ratio [see Table E3 in this article’s Online Repository at www.jacionline.org]). A panel of cytokines was analyzed in samples of nasal lavage fluid from a subset of 80 COAST children, with approximately equal representation from each of the 4 MPGs. In general, proinflammatory cytokine production was greatest in the Moraxella MPG, followed by the Staphylococcus, Streptococcus and Dolosigranulum MPGs (see Fig E9 in this article’s Online Repository at www.jacionline.org).

We next tested whether the association between trajectory C and asthma was mediated via viral wheezing illnesses or allergic sensitization in early life. To test this, all 3 variables (trajectory, early wheezing illness, and aeroallergen sensitization) were included as outcomes in multivariable models with asthma diagnosis at various time points. The association between trajectory C and asthma diagnoses at ages 6 to 13 was partially ablated with adjustment for both early aeroallergen sensitization (allergen-specific IgE level > 0.35 kU/L by age 2 years) and number of early-life wheezing illnesses up to age 3 (see Table E4 in this article’s Online Repository at www.jacionline.org). However, trajectory C remained a statistically significant predictor for asthma diagnosis at ages 11 and 13, suggesting that the

### TABLE I. Demographic characteristics of children in the 4 nasal microbiome trajectories

| Variable                      | A (n = 79) | B (n = 43) | C (n = 26) | D (n = 135) | P value |
|-------------------------------|------------|------------|------------|-------------|---------|
| Sex (% male)                  |            |            |            |             |         |
| Exclusive breast-feeding (%)  | 51%        | 51%        | 69%        | 59%         | .30     |
| Dog in home at birth (%)      |            |            |            |             |         |
| Cat at home at birth (%)      |            |            |            |             |         |
| Maternal asthma ever (%)      |            |            |            |             |         |
| Mother’s education (≥3 years of college) (%) | | | | | .91 |
| Household income ≥ $50,000 (%) | 57% | 68% | 62% | 54% | .43 |

Association analyses were conducted by using Fisher exact tests for categoric variables, whereas Kruskal tests were conducted for continuous variables across all trajectories.

![FIG 3. Association of nasal microbiome trajectories with the frequency of wheezing illnesses](image-url)

TABLE I. Demographic characteristics of children in the 4 nasal microbiome trajectories

- **Trajectory A**: (n = 79)
- **Trajectory B**: (n = 43)
- **Trajectory C**: (n = 26)
- **Trajectory D**: (n = 135)
microbiome trajectory may be acting via mechanisms not fully captured by wheezing illnesses or early-life aeroallergen sensitization.

The trajectories were robust to modifications in their derivation. We reproduced trajectories by using (1) only routine samples within the first 6 months of life (see the Online Data Supplement) or (2) only healthy samples. Both analyses yielded trajectories that were very similar to the originals (see Table E5 in this article’s Online Repository at www.jacionline.org), with similar associations with most asthma outcomes ($P < .05$ for all generalized linear model associations of asthma at age 8, 11, or 13 years with approximately trajectory C).

**DISCUSSION**

Developmental patterns of microbiome composition in the gut and skin can influence local immune function and the risk of developing allergic diseases.35-37 Similarly, we hypothesized that the developmental trajectory of the airway microbiome influences the risk of developing wheezing illnesses and asthma. Children in the COAST study could be separated into 4 developmental trajectories, each characterized by nasopharyngeal samples in the first 4 to 6 months of life as being dominated by a distinct bacterial taxon. In particular, trajectory C, characterized by early *Staphylococcus* colonization, was linked with a higher frequency of asthma at each scheduled assessment ($P$ values were obtained by using the chi-square test across all trajectories (top, in black) or post hoc Bonferroni-corrected comparisons for trajectory C versus all other trajectories (A + B + D [bottom, in purple]). Nasal microbiome trajectory C had a higher proportion of children with a persistent asthma phenotype than the other trajectories did (B) (trajectory C vs the other trajectories; $P = .08$).

Previous observational studies have provided information on temporal changes in composition of the airway microbiome in early life, and both community composition and maturation of the microbiome have been related to more frequent respiratory illnesses. In a study of 60 healthy children sampled several times (at the ages of 1.5, 6, 12, and 24 months) during the first 2 years of age, initial colonization with *Haemophilus*, *Streptococcus*, or *Staphylococcus* communities was associated with more frequent respiratory illnesses and was relatively unstable.16 In contrast, microbial communities associated with *Moraxella* and Corynebacterium/Dolosigranulum in the first few months were more stable. Our findings were similar in that trajectory B had the most stable composition, with *Moraxella* MPG detected most often at all ages tested.

The relationship between wheezing illnesses and *Staphylococcus* appears to be age dependent. Our study and others found that *Staphylococcus* was more prevalent in secretions from healthy young infants and was less likely to be detected in the first year of life during periods of illness. On the other hand, trajectory C, characterized by the *Staphylococcus* MPG, was associated with increased wheezing by age 3 years. To reconcile these findings, it is important to consider that the *Staphylococcus* MPG was predominant in trajectory C only for the first 6 months of life in COAST, and thereafter, *Moraxella* was the most common MPG. Accordingly, trajectory C was associated with fewer illnesses during the first year, followed by the highest frequency of illnesses during years 2 and 3. Teo et al also found that the negative association between *Staphylococcus* MPG and respiratory illness attenuated over time. Similarly, Bosch et al reported that early predominance of *Staphylococcus* transitioning to *Moraxella* was related to increased frequency of respiratory illnesses in a birth cohort study. Notably, nasal *S aureus* has also been related to asthma and bronchial hyperresponsiveness in children and wheeze in children and adults.

There are several potential mechanisms that could link *S aureus* colonization to childhood asthma. First, *S aureus* can produce superantigens that are potent stimulators of proinflammatory T-cell responses, and can promote type 2 inflammation by directly...
activating mast cells, as well as by inducing thymic stromal lymphopoietin. However, analysis of nasal cytokines did not indicate that the \textit{S aureus} MPG was associated with increased thymic stromal lymphopoietin level or a greater inflammatory milieu in well infants. Alternatively, staphylococci can produce toxins that can enhance viral replication, and this effect could lead to increased viral wheezing illnesses. Furthermore, \textit{S aureus} quorum sensing systems (agr) sense self-produced peptides and upregulate the production of toxins, providing a mechanism for enhanced virulence when \textit{S aureus} is present in higher quantities. On the skin, \textit{S aureus} colonization is closely linked to epithelial barrier dysfunction and disease activity in atopic dermatitis and is furthermore associated with a greater risk of sensitization and allergy to foods. Accordingly, trajectory C was linked to early allergen sensitization and allergic rhinitis in COAST. Alternatively, considering that \textit{Staphylococcus} is a predominant organism in the neonatal airway, trajectory C could indicate delayed maturation of the nasal microbiome. Delayed maturation of the microbiome could in turn delay development of airway mucosal immunity and hence lead to more frequent infections.

Detection of pathogen-dominated microbial communities (\textit{Moraxella}, \textit{Streptococcus}, \textit{Haemophilus}, and viruses) have previously been related to acute wheezing illnesses and...
to childhood asthma at age 5 years. Similarly, in COAST we found that both RV-associated illnesses and the presence of illness-associated bacteria (especially Moraxella) in nasopharyngeal samples, especially those collected in the second and third years of life, were predictive of persistent childhood asthma. Dumas et al found that a severe bronchiolitis profile characterized by eosinophilia and RV infections is associated with a Moraxella- or Haemophilus-dominated nasopharyngeal microbiota. Conversely, Rosas-Salazar et al found that copresence of Lactobacillus during RSV infections may be protective against childhood wheeze. These associations suggest that bacterial microbiota during health and disease may influence susceptibility to frequent early-life respiratory infectious illnesses, leading to inflammatory and/or structural changes and entrenchment of asthma. Furthermore, it is possible that there are 2 distinct mechanisms linking the early-life microbiome to asthma: a developmental trajectory that is related to early colonization with Staphylococcus and a second mechanism related to respiratory pathogens (Moraxella, Streptococcus, Haemophilus, and RV) during periods of illness.

Strengths of this study included intensive sampling of the nasal microbiome and virome in the first 2 years of life, which enabled analyses both of microbiome assembly during routinely observed states and during illness-related perturbations. In addition, COAST participants have been evaluated for asthma at regular states and during illness-related perturbations. In addition, analyses both of microbiome assembly during routinely observed microbiome and virome in the first 2 years of life, which enabled microbiota dynamics uncover a critical window for interplay of pathogenic bacteria and viruses with early-life respiratory infectious illnesses, leading to inflammatory and/or structural changes and entrenchment of asthma. Furthermore, it is possible that there are 2 distinct mechanisms linking the early-life microbiome to asthma: a developmental trajectory that is related to early colonization with Staphylococcus and a second mechanism related to respiratory pathogens (Moraxella, Streptococcus, Haemophilus, and RV) during periods of illness.

Clinical implication: Identifying factors that promote early colonization with S aureus may lead to future interventional studies to prevent childhood asthma.

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