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Distinct nasal airway bacterial microbiotas differentially relate to exacerbation in pediatric patients with asthma

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Abstract

Background: In infants, distinct nasopharyngeal bacterial microbiotas differentially associate with the incidence and severity of acute respiratory tract infection and childhood asthma development.

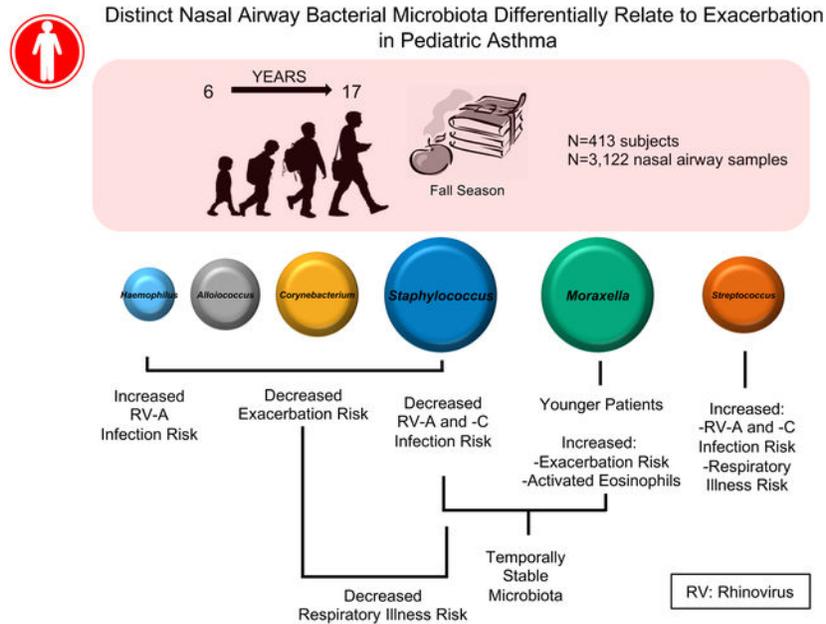
Objective: We hypothesized that distinct nasal airway microbiota structures also exist in children with asthma and relate to clinical outcomes.

Methods: Nasal secretion samples (n = 3122) collected after randomization during the fall season from children with asthma (6–17 years, n = 413) enrolled in a trial of omalizumab (anti-IgE) underwent 16S rRNA profiling. Statistical analyses with exacerbation as the primary outcome and rhinovirus infection and respiratory illnesses as secondary outcomes were performed. Using A549 epithelial cells, we assessed nasal isolates of *Moraxella*, *Staphylococcus*, and *Corynebacterium* species for their capacity to induce epithelial damage and inflammatory responses.

Results: Six nasal airway microbiota assemblages, each dominated by *Moraxella*, *Staphylococcus*, *Corynebacterium*, *Streptococcus*, *Alloiococcus*, or *Haemophilus* species, were observed. *Moraxella* and *Staphylococcus* species–dominated microbiotas were most frequently detected and exhibited temporal stability. Nasal microbiotas dominated by *Moraxella* species were associated with increased exacerbation risk and eosinophil activation. *Staphylococcus* or *Corynebacterium* species–dominated microbiotas were associated with reduced respiratory illness and exacerbation events, whereas *Streptococcus* species–dominated assemblages increased the risk of rhinovirus infection. Nasal microbiota composition remained relatively stable despite viral infection or exacerbation; only a few taxa belonging to the dominant genera exhibited relative abundance fluctuations during these events. *In vitro*, *Moraxella catarrhalis* induced significantly greater epithelial damage and inflammatory cytokine expression (IL-33 and IL-8) compared with other dominant nasal bacterial isolates tested.

Conclusion: Distinct nasal airway microbiotas of children with asthma relate to the likelihood of exacerbation, rhinovirus infection, and respiratory illnesses during the fall season.

Graphical Abstract



Keywords

Microbiota; *Moraxella species*; *Staphylococcus species*; *16S rRNA*; *airway*; *asthma*; *exacerbation*; *rhinovirus*

Viral respiratory tract infections have long been recognized as important contributors to wheezing illnesses and asthma exacerbations; however, there is growing interest in the role of the airway microbiome and its potential to modulate these events in children with asthma. ¹ Bisgaard et al² demonstrated that detection of *Streptococcus pneumoniae*, *Moraxella catarrhalis*, or *Haemophilus influenzae* in nasopharyngeal samples obtained at 1 month of age was linked to increased risk for asthma at 5 years of age. The Childhood Asthma Study, a prospective cohort of infants (n = 234), examined nasopharyngeal bacterial communities over the first year of life, including samples collected during periods with and without acute respiratory illness.³ Within this cohort, 6 compositionally distinct microbiotas, each dominated by *Moraxella*, *Staphylococcus*, *Corynebacterium*, *Streptococcus*, *Alloiococcus*, or *Haemophilus* species, were identified. Microbiotas dominated by *Moraxella*, *Streptococcus*, or *Haemophilus* species were associated with a significantly increased risk of virus-associated acute respiratory illness,³ and *Streptococcus* species-dominated microbiotas were also coassociated with increased risk of lower airway infection and subsequent childhood asthma development.

Prior acute sinus infection has been associated with significant relative enrichment of upper airway *Moraxella* species in children, and expansion of this genus in healthy (non-upper respiratory tract infection) samples predicts subsequent acute sinusitis⁴ and relates to airway

eosinophilia and bronchial inflammation in adults with asthma.⁵ Independently, upper airway detection of *S pneumoniae* has been associated with increased respiratory illness symptoms and moderate asthma exacerbations, particularly when co-detected with rhinovirus, whereas *M catarrhalis* and rhinovirus in combination, increased the likelihood of respiratory illness, asthma symptoms, or both compared with infection with rhinovirus alone.⁶ Thus the presence of specific bacterial genera and their coassociated microbiota assemblages strongly and reproducibly relate to respiratory illness, particularly in relation to viral infection. Given that viral respiratory tract infection is a risk factor for asthma exacerbation⁷ and that bacterial LPS has recently been shown to modulate viral stability,⁸ we hypothesized that compositionally distinct microbiotas exist in the nasal airways of children with asthma and are related to risk of viral infection and asthma exacerbation.

METHODS

Study design and clinical outcomes

This study followed 478 children with asthma (aged 6–17 years) from 8 urban clinics (Boston Chicago, Cincinnati, Dallas, Denver, Detroit, New York, and Washington, DC) participating in the Preventative Omalizumab or Step-up Therapy for Severe Fall Exacerbations (PROSE;) randomized controlled trial (see Table E1 in this article’s Online Repository at www.jacionline.org).⁹ Participants provided home-collected nasal secretion samples every 2 weeks throughout the 90-day fall outcome periods from one of 2 study years (September–December 2012 or 2013) for analysis in this study (Fig 1). Sample collection and storage were standardized across all clinical sites. Participants with fewer than 3 high-quality microbiota profiles available for analyses were removed. See the Methods section in this article’s Online Repository at www.jacionline.org for details of sample acquisition, processing, and analyses. Asthma exacerbations were defined *a priori* as physician-prescribed use of systemic corticosteroids for asthma symptoms, hospitalization for asthma, or both.^{10,11} Rhinovirus infection (detection of rhinovirus) was assessed by using quantitative PCR and partial sequencing to identify viral strain type.¹²

Microbiota analyses

DNA from nasal secretion samples was extracted by using a modified cetyltrimethylammonium bromide–polyethylene glycol protocol, and the variable 4 (V4) region of the 16S rRNA gene was amplified, quantified, and sequenced on a NextSeq 500 (Illumina, San Diego, Calif); details are provided in the Methods section in this article’s Online Repository. Statistical analyses were conducted with R¹³ and QIIME¹⁴ software. Relationships between community composition in initially collected independent samples and a range of clinical and viral infection variables were assessed by using *adonis* in the *vegan* package¹⁵ in R software. Respiratory illnesses were defined as an increased symptom score compared with pretreatment baseline symptoms.¹⁶ Respiratory symptom scores were based on a 0- to 3-point severity score (absent, mild, moderate, or severe) for 5 different symptoms (runny nose, stuffy nose, sore throat, sneezing, and cough); scores ranged from a minimum of 0 to a maximum of 15.

Significant differences in taxon relative abundance were assessed by using a 3-model approach, as previously described.¹⁷ Samples were stratified based on the genus-level identity of the dominant taxon (ie, the taxon that exhibited the largest proportion of sequence reads in each sample). Co-occurrence networks of operational taxonomic units were constructed by using the SparCC¹⁸ and WGCNA¹⁹ packages in R software. Repeated-measures analyses (linear mixed effects and generalized estimating equations) used participant as the random effect. *P* values were adjusted for false discovery by using the Benjamini-Hochberg method; a *q* value of 0.15 was considered significant.

***In vitro* epithelial response studies**

Human alveolar epithelial cells (A549) were exposed to the sterile products of 72-hour biofilm cultures of nasal *M catarrhalis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Corynebacterium propinquum* isolates. Epithelial cell damage was assessed based on lactate dehydrogenase release and inflammatory gene expression by using quantitative PCR. Further information is available in the Methods section in this article's Online Repository.

RESULTS

Clinical and viral factors are related to nasal airway microbiota composition

Of the 3840 samples received, 3122 samples from 413 children provided a high-quality microbiota profile for analysis. An average of 7.5 samples per person were available, and samples were collected an average of 10.7 days apart (Fig 1). Clinical, demographic, and microbiological factors (see Table E2 in this article's Online Repository at www.jacionline.org) measured during the 3-month postrandomization observation period were assessed for relationships with nasal airway microbiota composition (β -diversity). Using the first postrandomization sample (baseline sample) available from each study participant for analysis (Fig 1, blue bars), we determined that variance in nasal airway microbial composition (β -diversity) was significantly associated with the dominant bacterial genus present, study site, age, eosinophil cationic protein (ECP) concentration in nasal airway secretions at randomization, total number of rhinovirus infections per participant, and samples in which rhinovirus was detected (rhinovirus infection samples; $P < .05$ for all, permutational multivariate ANOVA; see Table E2). In contrast, microbiota composition at baseline was not significantly related to respiratory illness symptoms at the time of sample collection ($R^2 = 0.048$, $P = .067$), treatment group (placebo, omalizumab, or inhaled corticosteroid boost: $R^2 = 0.008$, $P = .079$; see Table E2), or future exacerbations in the outcome period ($R^2 = 0.0043$, $P = .101$). These observations were supported by using a bootstrapped analysis (ie, repeatedly sub-sampling a single randomly selected sample for each participant; 500 times; see Table E2 and additional information in the Methods section in this article's Online Repository).

Although overall nasal microbiota composition at baseline was not related to exacerbation in the outcome period, this did not preclude the possibility that specific taxa could predict future exacerbations. To examine this possibility, we performed comparative and machine learning analyses to determine relationships between baseline bacterial taxa relative abundance and asthma exacerbations in the outcome period. A number of distinct

Corynebacterium and *Acinetobacter* taxa were depleted at baseline in children who went on to experience an exacerbation (see Fig E1, A, in this article's Online Repository at www.jacionline.org). Elastic nets analysis identified distinct *Moraxella* and *Staphylococcus* taxa that were predictive of exacerbation or nonexacerbation outcomes (see Fig E1, B), indicating that specific species or strains within these genera might relate to subsequent exacerbation susceptibility.

Taxa associated with exacerbation and rhinovirus infection coassociate in bacterial networks

The bacterial genus most abundant in each sample (the dominant taxon) explained a large proportion of variability in microbiota composition (Fig 2, A, and see Table E2), indicating that a gradient of microbiota compositions punctuated by the presence of distinct dominant bacterial genera exist in the nasal airways of children with asthma. The majority of samples in our study were dominated by either *Moraxella* species (n = 1058 [33.9%]) or *Staphylococcus* species (n = 953 [30.5%]); the remaining samples were dominated by *Streptococcus* (n = 263 [8.4%]), *Alloiococcus* (n = 261 [8.4%]), *Corynebacterium* (n = 153 [4.9%]), or *Haemophilus* (n = 135 [4.3%]) species or other genera (n = 299 [9.6%]; Fig 2, B). The specific taxa characteristically dominating each of the 6 most common microbiotas were also amongst those significantly enriched or depleted in samples with rhinovirus infection (vs no infection) or respiratory illness (vs none) or children who experienced asthma exacerbations (vs no exacerbations) during the outcome period (see Figs E2 and E3 in this article's Online Repository at www.jacionline.org). This suggested that distinct nasal airway microbiotas dominated by different bacterial genera exist in children with asthma and that taxa within these microbiotas relate to the primary (asthma exacerbation) and secondary (rhinovirus infection and respiratory illness) clinical outcomes in this cohort.

To identify specific bacterial taxa that consistently coassociate within the nasal airway microbiota, we next applied network analysis to microbiota profiles generated from all samples (Fig 3 and see Table E3 in this article's Online Repository at www.jacionline.org). Notably, dominant *Moraxella* taxa consistently coassociated almost exclusively with other *Moraxella* taxa, including several that were enriched in participants who experienced exacerbations (Fig 3 and see Table E4 in this article's Online Repository at www.jacionline.org). In contrast, the dominant taxa in each of the other nasal airway microbiotas formed bacterial networks consisting of both phylogenetically similar and distinct genera, often including the same specific taxa enriched in rhinovirus-negative and nonexacerbation samples.

Nasal microbiotas are associated with clinical outcomes

We next tested whether the 6 most prevalent nasal airway microbiota assemblages covaried with primary (asthma exacerbation) and secondary (rhinovirus infection and respiratory illness events) clinical outcomes. Using all longitudinal samples (n = 3122) and generalized estimating equations, we noted that asthma exacerbation, rhinovirus infection, and respiratory illness events varied significantly across the 6 nasal airway microbiotas. Children who experienced 1 or more exacerbations in the outcome period were more likely to possess *Moraxella* species-dominated microbiotas (relative risk [RR] = 1.66; $q = 0.04$) in their

longitudinally collected samples. This observation was no longer significant after adjustment for participant's age (adjusted RR = 1.41, $q = 0.22$). However, it should be noted that age was related to microbiota composition in our cohort (see Table E2) and that younger children were more likely to possess *Moraxella* species–dominated microbiotas (Table I and see Fig E4 in this article's Online Repository at www.jacionline.org). Although exacerbation was more likely to be associated with *Moraxella* species–dominated microbiotas, rhinovirus infection was not, suggesting that microbiotas dominated by species of *Moraxella* might promote asthma exacerbation irrespective of rhinovirus infection. This is supported by our observation that ECP concentrations in nasal secretions (a marker of activated eosinophils) measured at randomization were significantly greater in *Moraxella* species–dominated samples collected closest to the ECP measurement (Table I and see Fig E4) and that ECP concentrations did not significantly differ in rhinovirus-infected or uninfected samples dominated by *Moraxella* species ($\beta = 0.12$, $P = .40$). In longitudinally collected samples, children who experienced 1 or more exacerbations were less likely to have nasal airway microbiotas dominated by *Haemophilus* species (RR = 0.41, $q = 0.04$), a finding that remained consistent after adjustment for age (adjusted RR = 0.39, $q = 0.07$; see Table E5 in this article's Online Repository at www.jacionline.org). Similarly, microbiotas dominated by *Alloiococcus*, *Corynebacterium*, or *Staphylococcus* species were also less likely to be observed in longitudinally collected samples from children who experienced 1 or more exacerbations in the outcome period (RR = 0.61, $q = 0.14$; RR = 0.44, $q = 0.12$; and RR = 0.70, $q = 0.14$, respectively).

Distinct nasal microbiotas relate to viral infection outcomes

Using all available samples ($n = 3122$), we found that samples with rhinovirus infection, specifically those samples with rhinovirus A detected, were less likely to exhibit a *Staphylococcus* species–dominated microbiota (RR = 0.84, $q = 0.05$ and RR = 0.75, $q = 0.05$, respectively). In addition, participants with a greater number of virus-positive samples were also less likely to possess a *Staphylococcus* species–dominated nasal airway microbiota (RR = 0.89, $q = 0.03$). Conversely, rhinovirus infection, specifically rhinovirus A, or respiratory illness events were more likely to occur in children with a *Streptococcus* species–dominated microbiota (RR = 1.54, $q < 0.01$ [rhinovirus infection]; RR = 1.70, $q = 0.03$ [rhinovirus A infection]; and RR = 1.78, $q < 0.01$ [respiratory illness]). However, asthma exacerbations were not significantly associated with the frequency of *Streptococcus* species–dominated microbiotas in this cohort.

We hypothesized that interactions between rhinovirus and the upper airway microbiota relate to clinical outcomes, specifically to asthma exacerbation and respiratory illness events. To test this, we determined the distribution of distinct microbiotas in rhinovirus-infected samples ($n = 969$), rhinovirus-infected samples with a concomitant respiratory illness event ($n = 245$), or rhinovirus-infected samples with a concomitant asthma exacerbation event ($n = 54$; see the Methods section in this article's Online Repository for details; $n = 1268$ total samples). Rhinovirus infection with asthma exacerbation samples exhibited a distinct distribution of microbiotas ($P < .001$, generalized estimating equations ANOVA; see Fig E5 in this article's Online Repository at www.jacionline.org). Consistent with our earlier taxonomic analyses, *Corynebacterium* and *Alloiococcus* species–dominated microbiotas

were less likely to be detected in rhinovirus infection with exacerbation samples. However, comparisons across the 6 individual microbiotas did not achieve statistical significance (see Fig E5), likely because of the limited number of rhinovirus infection with asthma exacerbation samples available for analysis ($n = 54$). Rhinovirus infections with respiratory illness (compared with rhinovirus infection without asthma exacerbation or respiratory illness symptoms) also trended toward differences in microbiota distributions ($P = .087$, generalized estimating equations ANOVA). Specific comparisons between microbiota states indicated that *Streptococcus* species–dominated microbiotas and rhinovirus infection significantly increased the RR of respiratory illness ($RR = 1.82$, $P = .014$; see Fig E5).

***Staphylococcus* and *Moraxella* species–dominated microbiotas exhibit temporal stability**

Leveraging the longitudinal nature of sample collection in this trial, we next examined microbiota stability and determined that *Moraxella* and *Staphylococcus* species–dominated microbiotas were most frequently maintained in the nasal airways over time (Fig 4). These observations were consistent irrespective of whether analysis was performed on samples collected within a defined time period (7–13 days between sample acquisition) or only the first 3 samples per participant were considered (to account for possible sample collection timing biases, see Fig E6 in this article’s Online Repository at www.jacionline.org). Multiple factors differentiated subjects who were stably colonized by a *Moraxella* or *Staphylococcus* species–dominated nasal microbiota (defined as >50% of a subject’s longitudinal samples dominated by *Moraxella* or *Staphylococcus* species). Children with stable *Moraxella* species colonization were more likely to have viral asthma exacerbations and a greater number of rhinovirus infections and respiratory illnesses during the study period. Children with stable *Staphylococcus* species colonization were typically older and had greater lung function values (likely colinear with age), a longer duration of asthma, and greater body mass index (see Table E6).

We next used longitudinal samples from participants experiencing an asthma exacerbation in the outcome period ($n = 54$ participants and $n = 498$ samples) to examine the effect of exacerbation on both microbiota composition stability and bacterial taxon dynamics. Many participants (50%) exhibited compositionally stable microbiotas (defined as a standard deviation of PC1 < 0.25) dominated primarily (>50% of samples within a subject) by *Moraxella* (15/54 [28%]) or *Staphylococcus* (6/54 [11%]) species, despite exacerbation or rhinovirus infection (see Fig E7, A, in this article’s Online Repository at www.jacionline.org). At the taxon level, the majority of taxa exhibited consistent relative abundance over the period of observation; however, a small number of specific taxa, primarily belonging to the 6 dominant genera, exhibited temporal fluctuations in relative abundance during rhinovirus infection or exacerbation events (see Fig E7, B).

Differential effect of dominant nasal bacteria on *in vitro* airway epithelial responses

Spurred by the observation that nasal airway microbiotas dominated by distinct bacterial respiratory pathogens differentially associate with clinical outcomes in our cohort, we hypothesized that the bacterial species dominating these microbiotas exert differing effects on the airway epithelium. Using enrichment media, we isolated strains of *M catarrhalis*, *S epidermidis*, *S aureus*, and *C propinquum* from nasal secretions of children with asthma and

identified those exhibiting greater than 97% sequence identity to dominant bacterial taxa identified in this study (see the Methods section in this article's Online Repository). Cell-free sterile products of biofilm cultures (which mimic physiologic features of mucosal-adherent bacteria) of each of these isolates and *Lactobacillus sakei* (previously found to protect against nasal airway infection)²⁰ were used to stimulate airway epithelial cell-line cultures *in vitro* before assessment of epithelial damage (lactate dehydrogenase levels in supernatants) and inflammatory gene expression. Compared with *S epidermidis* or *C propinquum*, the cell-free products of *M catarrhalis* isolates consistently increased epithelial damage ($P < .01$, Wilcoxon test) and gene expression of the proinflammatory cytokines IL-8 and IL-33 ($P < .03$, Wilcoxon test; Fig 5), as well as MUC5AC, CXCL10, and the epithelial tight junction protein occludin (see Fig E8 in this article's Online Repository at www.jacionline.org). Of note, in comparison with *S epidermidis* or *C propinquum*, *S aureus* also increased expression of IL-8 ($P = .003$ and $P = .002$, respectively, Wilcoxon test; Fig 5) in addition to CXCL10 and occludin ($P < .05$, Wilcoxon test; see Fig E8). Hence *M catarrhalis* and *S aureus*, but not *S epidermidis* or *C propinquum*, appear capable of promoting relatively increased epithelial damage and inflammation *in vitro*, offering a potential mechanism by which nasal microbiotas dominated by these distinct species might differentially relate to clinical outcomes in this cohort.

DISCUSSION

Our longitudinal analyses of the nasal airway bacterial microbiotas of more than 3000 samples from more than 400 children with asthma in the PROSE trial identified distinct microbiotas associated with risk of asthma exacerbation, rhinovirus infection, and respiratory illness. Omalizumab therapy, which modulates IgE responses, successfully reduced fall exacerbations in children with asthma¹⁰; however, it did not significantly alter nasal airway microbiota composition, raising the possibility that the nasal microbiota remains largely unaffected by treatments targeting specific features of asthma-associated immune dysfunction. The persistence of pathogenic nasal airway microbiotas, particularly those dominated by *Moraxella* species, which more frequently occurred in younger children, were associated with increased eosinophil activation and asthma exacerbations. This helps explain several key questions in the field: why some children with asthma are "exacerbation prone," why some experience exacerbations in the absence of viral infection, and why asthma symptoms frequently recur after cessation of anti-inflammatory treatments.

Perturbations to the upper respiratory microbiota have been described in children^{2,3,6,21} and adults^{5,22-24} with asthma, and recent evidence indicates that upper airway *Moraxella* species are also detected in the lower airways of patients with asthma.^{5,25} Consistent with our observations, the relative abundance of upper respiratory *Moraxella* species has been positively correlated with markers of eosinophilic inflammation (in both bronchoalveolar lavage fluid and blood) and negatively correlated with *Corynebacterium* species.⁵ Although specific mechanisms by which microbiotas of the upper airways contribute to lower airway inflammation and asthma exacerbations remain unclear, a growing body of evidence suggests that the upper airway microbiota can serve as an inflammatory trigger and/or the source of pathogenic microbes in the lower airway of those with chronic respiratory disease. Indeed, although many taxa detected in the nasal airway microbiota contribute to the relative

temporal stability of these assemblages, the relative abundance of a small number of discrete taxa dynamically shift in parallel with asthma exacerbation and rhinovirus infection events, suggesting that these organisms may contribute to clinical outcomes.

Approximately 20% to 30% of asthma exacerbations in children are not associated with viral infection, and their cause remains enigmatic.²⁶ Our observations provide evidence that some asthma exacerbations might not be related to viral infection but rather to pathogenic bacterial activities, specifically *Moraxella* species–dominated microbiota. Our identification of *M catarrhalis* as the dominant species in nasal airway microbiotas of children who experience a greater frequency of exacerbation and subsequent *in vitro* evidence for the capacity of strains of this species to induce epithelial damage and increase IL-8 and IL-33 expression, even in the absence of rhinovirus coinfection, support a role for this species in asthma pathogenesis. *M catarrhalis* is an opportunistic human respiratory pathogen that encodes a range of proteins and ligands that promote its adherence and invasion of epithelial cells and can, through induction of innate immune responses and complement evasion, result in tissue destruction and promote long-term persistence on the mucosal surface.²⁷ Future studies using shotgun metagenomic and paired RNA sequencing analyses will be required to determine the extent of virulence genes and their expression in the airway microbiota. Such information could lead to novel treatment or vaccination strategies to prevent nasal airway colonization by pathogenic genera and reduce exacerbation risk in children with asthma.

The microbiotas of children who did not experience asthma exacerbations were more likely to be *Alloiococcus*, *Haemophilus*, *Corynebacterium*, or *Staphylococcus* dominated; reduced risk of respiratory illness was also associated with the latter 2 microbiotas. These observations are largely consistent with the findings of Teo et al³ in the Childhood Asthma Study, who noted that infants whose nasal airways were colonized by bacterial communities dominated by *Alloiococcus*, *Corynebacterium*, or *Staphylococcus* species were at significantly lower risk of acute respiratory tract infection. Our observation that the biofilm products of *S epidermidis* and *C propinquum* (identified as dominant members of these protective microbiota) induced less epithelial damage and IL-8 and IL-33 expression compared with *M catarrhalis* suggests that distinct nasal airway microbiota dominated by these species can differentially modulate mucosal integrity and immunity in a manner that alters susceptibility and the severity of respiratory illness in children with asthma.

It should be noted that the Childhood Asthma Study reported that although *Haemophilus* species–dominated communities were infrequently detected in the infant nasopharynx (1.2% of population studied), these infants were at extremely high risk of acute respiratory illness.³ In our study of older children with established asthma, *Haemophilus* species–dominated communities were also less frequently detected, but their presence was associated with reduced risk of exacerbation that was plausibly explained by differences in age and airway development that exists between infants and children, bacterial strain differences, or increased *H influenzae* vaccination in this older population. Related to this, we did note a significant relationship between the nasal airway microbiota and the participant’s age, with *Moraxella* species–dominated microbiotas more frequently detected in younger participants, indicating that younger children with asthma who possess a *Moraxella* species–dominated nasal airway microbiota might represent a particular subset of patients at heightened risk for

exacerbation. Consistent with previous reports,^{6,28} rhinovirus infection or respiratory illness samples were more likely to be associated with *Streptococcus* species–dominated microbiotas but, contrary to those reports, not with asthma exacerbations in our cohort. When focusing on rhinovirus infections, we noted an increased risk of asthma exacerbation in the presence of *Streptococcus* species, but this finding did not reach significance.

Despite our large sample size, there are a few study limitations to consider. Our study did not include prerandomization samples or nasal airway samples from children without asthma, limiting our ability to determine whether the microbiotas we describe characterize children without asthma at baseline. Also, the findings presented in this study derive from children selected on the basis of specific entry criteria and might be generalizable primarily to children with severe asthma living in low-income urban environments. Furthermore, fungal communities can also play a significant role in the nasal airway microbiotas of children with asthma; however, they have yet to be characterized in this population. We also note that other factors, such as host genetics, epigenetics, preterm birth,²⁹ non-rhinovirus viral infections, allergens, or microbial products, might also be related to observations made in this study. In addition, the V4 region of the 16S rRNA gene used to determine bacterial taxonomy is frequently unable to discriminate between bacterial species or strains whose V4 region is identical. This is exemplified by our finding that both *S epidermidis* and *S aureus* belong to the same dominant *Staphylococcus* taxon; however, each elicits a distinct and opposing effect on epithelial integrity and inflammatory gene expression. This could explain the apparent contradiction that although *Staphylococcus* species–dominated communities (possibly *S epidermidis* species–dominated communities) were protective against asthma exacerbation in our overall cohort, many of the children who provided acute asthma exacerbation samples also exhibited *Staphylococcus* species–dominated (possibly *S aureus* species–dominated) microbiotas. Indeed, a recent report demonstrated that *S aureus* directly induces type 2 cytokine expression in nasal polyp tissue from older patients with chronic rhinosinusitis, which was not recapitulated upon infection with *S epidermidis*,³⁰ suggesting these distinct *Staphylococcus* species induce differential inflammatory responses.

In conclusion, this study identifies distinct nasal airway microbiotas that are differentially related to the risk for asthma exacerbation, rhinovirus infection, and respiratory illnesses. Our data form a foundation for more in-depth investigations to determine how distinct nasal airway microbiomes and, more specifically, active members of these assemblages interact with the host mucosa to promote or protect from exacerbations in children with asthma. Moreover, microbiome-based identification of children with asthma at heightened risk for exacerbation could lead to targeted strategies to promote appropriate nasal airway mucosal colonization and potentially reduce exacerbation risk. Additional studies are necessary before such strategies could be considered for clinical implementation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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We thank the PROSE participants for their contribution to this study. Sequence data are available on the European Nucleotide Archive under accession number PRJEB25616.

Abbreviations used

ECP	Eosinophil cationic protein
PROSE	Preventive Omalizumab or Step-Up Therapy for Severe Fall Exacerbations
RR	Relative risk
V4	Variable 4

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Key messages

- Children with asthma (age range, 6–17 years) who possess a *Moraxella* species–dominated nasal airway microbiota are typically younger and at increased risk of exacerbation.
- Nasal airway microbiotas dominated by *Staphylococcus* or *Corynebacterium* species were associated with reduced risk of exacerbation and respiratory illness events, whereas *Streptococcus* species–dominated microbiotas increased the risk of upper respiratory illnesses.
- *Moraxella* and *Staphylococcus* species–dominated microbiotas are stably maintained in the upper airways of children with asthma.

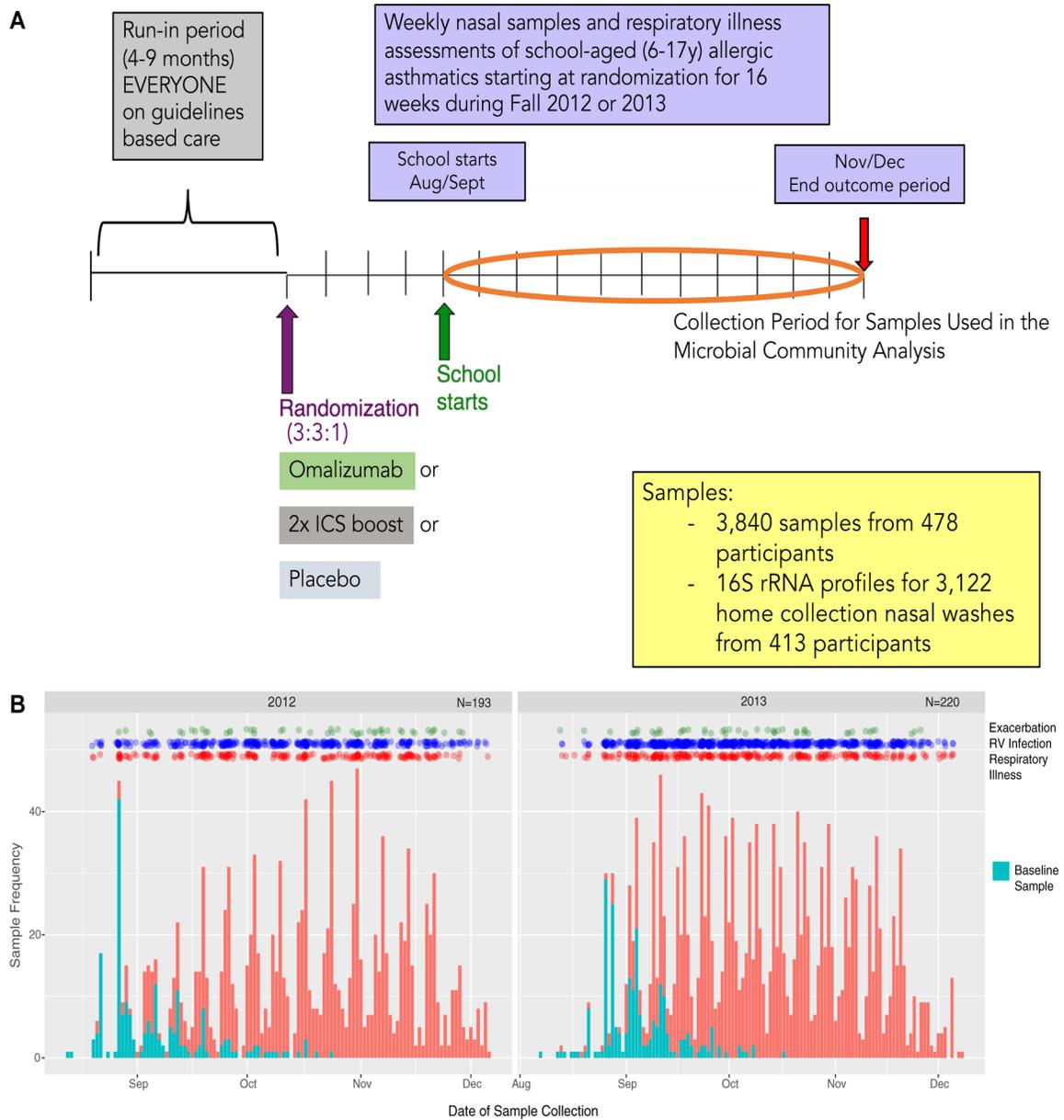


FIG 1. **A**, Study design and distribution of microbiota profiles from children in the PROSE study (10) used in the current study. *ICS*, Inhaled corticosteroid. **B**, Frequency and timing of sample collection. *Blue bars* depict the first postrandomization (baseline) sample collected from participants, and all subsequent longitudinal samples collected are indicated by *red bars*. *Green, blue, and red dots* indicate exacerbation, rhinovirus infection (RV), and respiratory illness events, respectively.

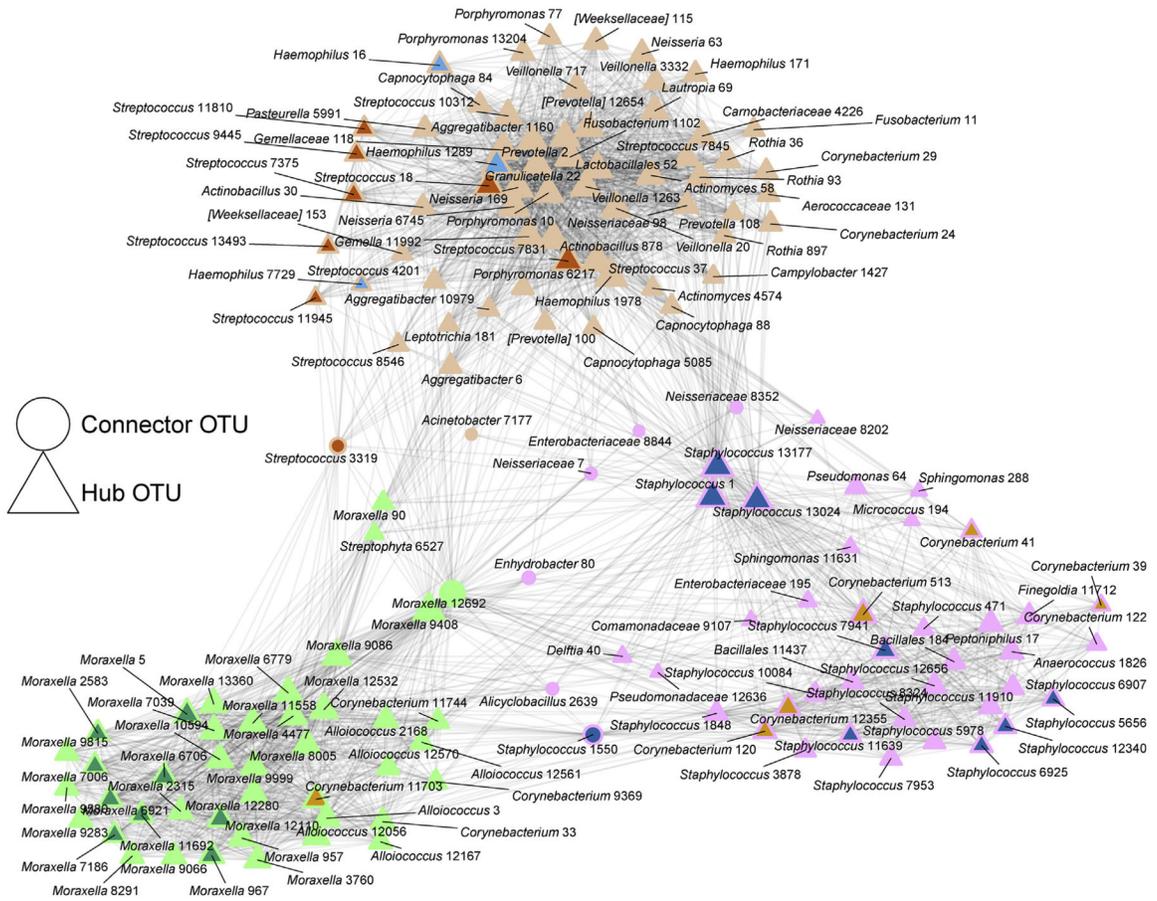


FIG 3. Network analysis identifies 3 distinct modules of coassociated nasal airway bacterial taxa. Taxa identified as differentially enriched in taxon comparisons of exacerbation versus nonexacerbation and rhinovirus infection versus non-rhinovirus infection comparisons are indicated and color coded according to the dominant microbiota colors defined in Fig 2. Hub operational taxonomic units (*OTUs*; triangles) exhibit greater intermodule connectivity, whereas connector OTUs (*circles*) exhibit a higher frequency of intramodule connectivity. The size of the node (triangles or circles) scales with the total number of connections with other OTUs. Genus classification and OTU numbers are indicated.

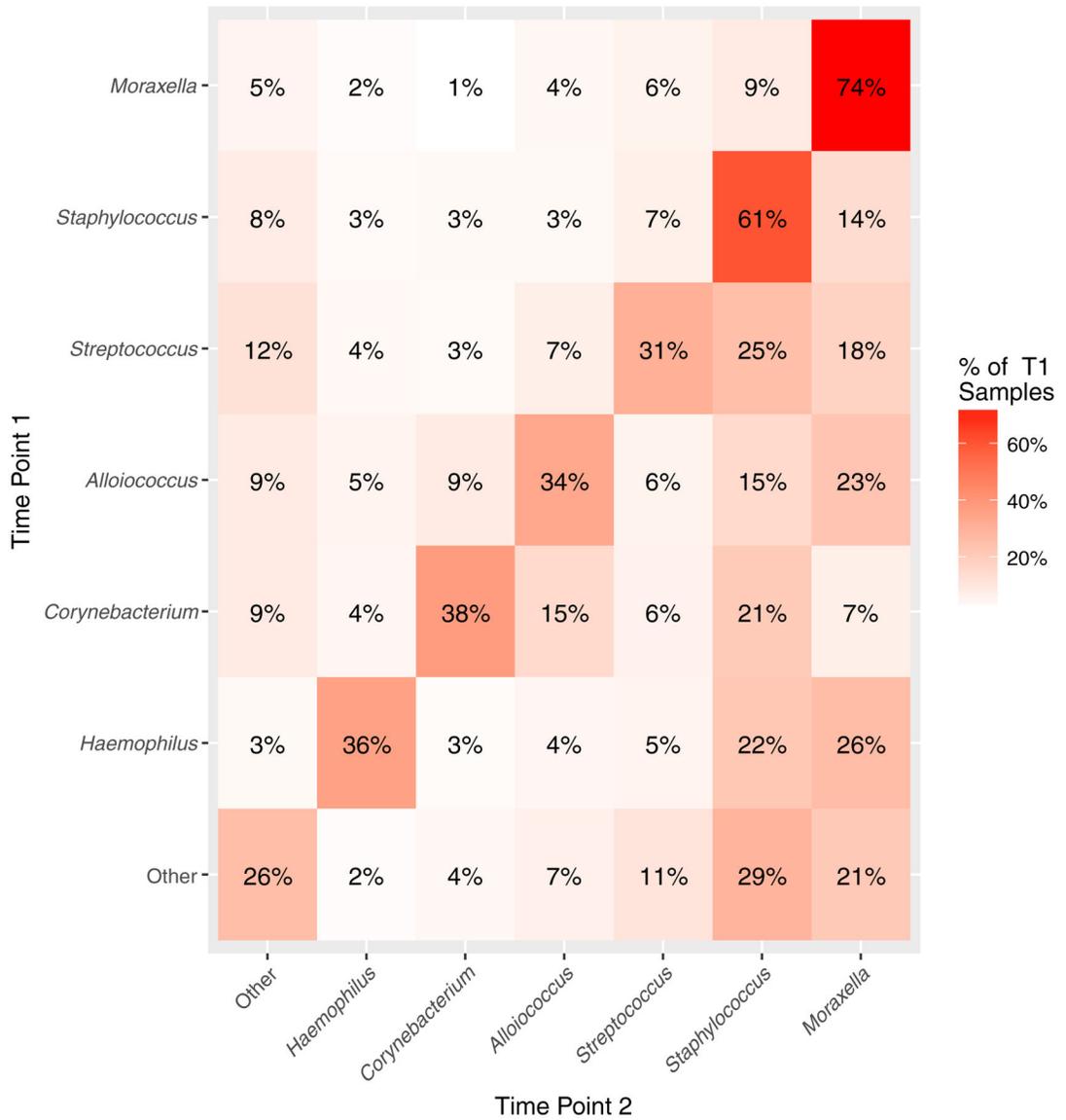


FIG 4. *Staphylococcus* and *Moraxella* species–dominated nasal airway microbiotas exhibit temporal stability in children with asthma. The heat map indicates the frequency with which a specific microbiota at a given time point (*y axis*) remains the same or transitions to a distinct microbiota assemblage in the subsequent patient sample (time point 2; *x-axis*). Frequencies of these events are provided within each square and proportions are indicated by the color intensity (eg, 74% of *Moraxella* species–dominated microbiotas remain *Moraxella* species–dominated in the subsequent sample). The diagonal represents transitions that resulted in maintenance of the same microbiota assemblage over time. Data were generated from longitudinally collected sample transitions ($n = 2709$) from all participants ($n = 413$).

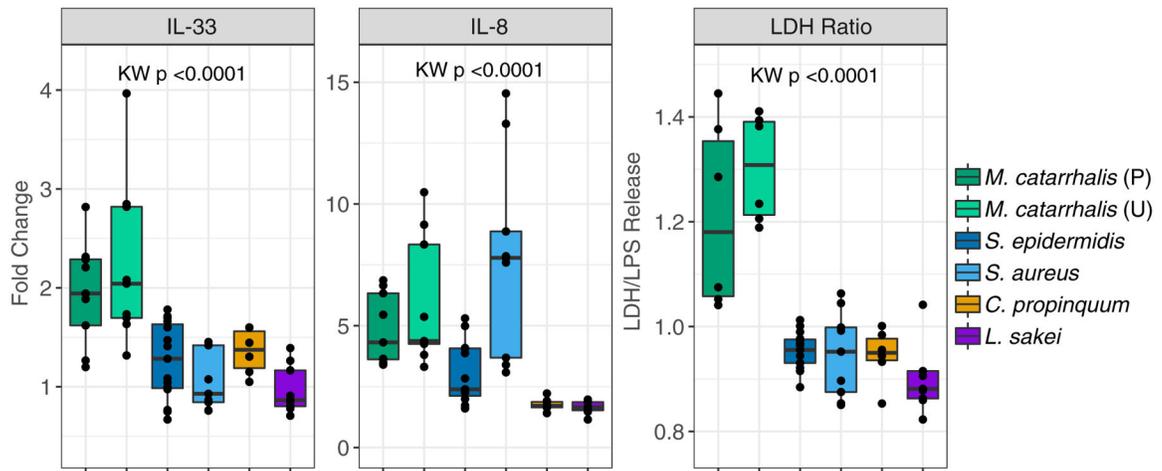


FIG 5. Biofilm-derived products of bacterial isolates dominating nasal airway microbiotas differentially influence epithelial responses *in vitro*. Comparative analysis of airway epithelial immune gene expression (relative to PBS) and lactate dehydrogenase (*LDH*) release (relative to LPS stimulation) after exposure to sterile biofilm supernatants of *Moraxella catarrhalis* (2 strains, see the Methods section in this article’s Online Repository for more information), *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Corynebacterium propinquum*. *M catarrhalis* strains consistently induce increased epithelial damage (*LDH*) and inflammation (*IL-8* and *IL-33*) compared with *S epidermidis*, *C propinquum*, and *Lactobacillus sakei*. Statistical significance was determined by using Kruskal-Wallis (*KW*) tests. Results were obtained from 2 or more independent experiments using 3 biological replicates.

TABLE I. Distinct nasal airway microbiotas differentially associate with exacerbation, viral outcomes, and clinical events

	Alloiococcus microbiotas		Corynebacterium microbiotas		Haemophilus microbiotas		Moraxella microbiotas		Staphylococcus microbiotas		Streptococcus microbiotas		Other microbiotas	
	RR	q Value*	RR	q Value	RR	q Value	RR	q Value	RR	q Value	RR	q Value	RR	q Value
Per-participant outcomes														
Child's age at randomization [†]	1.01	0.90	1.35	<0.01	1.07	0.69	0.84	< 0.01	1.05	0.32	0.96	0.69	0.97	0.69
FEV ₁ /FVC ratio at randomization [†]	1.01	0.68	0.97	0.68	1.02	0.69	0.99	0.69	1.00	0.69	1.02	0.68	0.98	0.68
Log(ECP) at randomization [†]	0.94	0.78	0.58 [§]	0.21	0.65	0.58	1.75	0.03 ^{//}	0.86	0.58	0.90	0.78	1.09	0.78
Exacerbation (participant) [‡]	0.61	0.14	0.44 [§]	0.12	0.41	0.04 ^{//}	1.66 [§]	0.04 ^{//}	0.70 [§]	0.14	1.22	0.42	1.23	0.40
No. of viral infections [‡]	1.05	0.63	0.88 [§]	0.32	1.01	0.96	1.10	0.10	0.89	0.03 ^{//}	1.06	0.48	1.00	0.96
No. of respiratory illnesses [‡]	1.05	0.92	0.97	0.92	1.03	0.92	1.00	0.99	0.90	0.29	1.15	0.29	1.07	0.51
Per-sample outcomes [‡]														
Any rhinovirus infection	0.96	0.75	0.94	0.75	1.26	0.38	1.09	0.38	0.84	0.05	1.54	< 0.01	0.80	0.22
Rhinovirus A infection vs rhinovirus negative	0.89	0.65	0.87	0.65	1.80	0.05	1.14	0.35	0.75	0.05	1.70	0.03	0.68	0.17
Rhinovirus B infection vs rhinovirus negative	1.29	0.39	0.85 [§]	0.50	0.87	0.67	1.13	0.39	0.89	0.39	1.34	0.39	0.65	0.26
Rhinovirus C Infection vs rhinovirus negative	0.96	0.85	1.22 [§]	0.80	1.26	0.80	0.94	0.80	0.79	0.14	1.63	0.14	1.08	0.80
Respiratory illness sample ^{//}	0.84	0.49	0.56	0.05	1.35	0.33	0.95	0.63	0.76	0.05	1.78	< 0.01	1.40	0.05

FVC, Forced vital capacity.

* q Values are P values adjusted for multiple comparisons by using a Benjamini-Hochberg false discovery rate across distinct microbiotas for each covariate of interest.

[†] All variables measured at randomization were associated solely with the first-collected microbiota sample, and generalized linear models using the initial sample were used with a binomial outcome.

[‡] Results use all data from samples collected from a subject throughout the outcome period, and generalized estimating equations using all longitudinal samples were used with a binomial outcome and an exchangeable correlation structure.

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§ Estimate changed by more than 10% after adjustment for age.

|| P value was no longer significant after adjustment for age. Significant differences are emphasized at *d* values of less than 0.15 in boldface.

¶ Respiratory illness occurred concurrently with sample collection.