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Original Article

Nasopharyngeal microbial profiles associated with the risk of airway allergies in early childhood

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Abstract *Background:* Airway microbiota may play an important role in regulating the immune response related to allergic respiratory diseases. A molecular-based approach was used to analyze the association between nasopharyngeal microbiota, serum immunoglobin (Ig)E levels, and childhood respiratory allergies.
Methods: Nasopharyngeal swabs were collected from children aged 36 months with three phenotypes, including allergic respiratory diseases plus atopy, atopy alone, and healthy controls for microbiome analysis using Illumina-based 16S rRNA gene sequencing.
Results: In total, 87 children were enrolled, including 36 with allergic respiratory diseases plus atopy, 21 with atopy alone, and 30 healthy controls. Proteobacteria (45.7%), Firmicutes (29.3%), and Actinobacteria (15.3%) were the most prevalent phyla in the study population. Compared with healthy controls, a lower Chao1 index was found in children with allergies ($P < 0.035$), indicating that bacterial richness was inversely associated with airway allergies.

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Additionally, in comparison with healthy controls, the genera *Acinetobacter*, *Moraxella*, *Asaia*, and *Rhodococcus* were more abundant and positively correlated with total serum IgE levels in children with allergies ($P < 0.01$), whereas the genera *Enterococcus* and *Rickettsia* were inversely correlated with total IgE levels, and also appeared to be negatively associated with airway allergies ($P < 0.01$).

Conclusions: The composition of the nasopharyngeal microbiota alteration may have an influence on childhood respiratory allergies. The inverse association between bacterial richness and allergies postulated that children living in a microbially hygienic environment may increase their risk of developing respiratory allergies.

Introduction

Allergic respiratory tract diseases, including asthma and allergic rhinitis, are the most prevalent childhood diseases and remain a major health issue for children.[1,2] Like other immune-mediated diseases, asthma or other allergies are complex diseases caused by the combined effects of genetic and environmental factors.[3] Genome-wide association studies in asthma patients have yielded several genes, such as *IL-13* and *FcεRI-β*, which are known to modify microbial pattern recognition receptors of the innate immune system.[4] Epidemiologic studies have shown that a number of environmental exposures are associated with early life events known to alter the microbiota, such as perinatal antibiotics, cesarean delivery, and formula feeding.[5] These studies indicate the importance of microbiota in gene—environment interactions in children with asthma and other allergic diseases.

Microbial communities interact with the mucosal immune system to maintain homeostasis in healthy airways. However, if microbiome dysbiosis (perturbation to the composition and function of the microbiota) occurs in the respiratory tract, it may induce an imbalance in the immune system, leading to the development of asthma or other inflammatory respiratory diseases.[6] Allergen sensitization, which can trigger $CD4^+$ T-helper type 2 (Th2) cell-mediated immune responses, is known to be an important factor in children's allergic diseases.[7] Thus, the interaction between microbiome dysbiosis and allergic reactions in response to allergen exposure plays an important role in allergic respiratory tract diseases.[8]

Previous studies have focused on identifying pathogenic microorganisms that are potentially associated with the occurrence of asthma; however, few studies have focused on the association between the composition of the airway microbiota and allergic respiratory tract diseases in early childhood.[9—11] The development of microorganism identification technologies from culture-dependent to molecular-based technologies has made further in-depth microbiome research possible.[12] These molecular-based techniques provide a more sensitive and culture-agnostic method of sampling microbes present in airway samples.[13]

A birth cohort study was conducted, and infants were prospectively examined for nasopharyngeal colonizing bacteria at specified time points. The nasopharynx, which lies between the nose, ear, sinuses, and lower respiratory tract, is a major source of secretions containing a diverse community of microbes.[14] To better understand the influence of the airway mucosal microbiome on respiratory allergies, we used molecular-based techniques to compare the nasopharyngeal microbial compositions between children with respiratory allergies and non-atopic healthy controls. The demographic features of the different groups were also assessed.

Methods

Study population

A cross-sectional study was designed to investigate the airway microbiota profiles of children with three phenotypes, including allergic respiratory diseases plus atopy, atopy alone, and non-atopic healthy controls. Children aged 36 months were enrolled, and nasopharyngeal swabs were collected for microbiome analysis. The physician-diagnosed phenotypes of allergic respiratory diseases, namely asthma or allergic rhinitis, were evaluated by the same pediatrician. Asthma was defined as doctor-diagnosed asthma, wheezing symptoms, or use of asthma medication during the last 12 months.[15] Allergic rhinitis was diagnosed as having sneezing, nasal congestion, itching of the eyes, or rhinorrhea other than respiratory tract infection during the previous 12 months.[16] A child with any of the above diseases was considered to have an allergic respiratory disease. Atopy was defined as immunoglobin (Ig)E sensitization (total IgE level $\geq$100 kU/L). A child was classified as having atopy alone if he/she had IgE sensitization (total IgE level $\geq$100 kU/L) without the above allergic respiratory diseases, history of food allergy, and atopic dermatitis, defined as a pruritic rash over the face and/or extensors with a chronic relapsing course. If a child had no allergic disease, IgE sensitization, or respiratory tract infection, he/she was considered a healthy control.

All the children in this study were from the Prediction of Allergens in Taiwanese Children study, a longitudinal birth cohort study launched in 2012 to investigate the epidemiology of bacterial colonization and factors associated with the development of allergic diseases. Women in the third trimester of pregnancy admitted at the Obstetrics Clinic of Keelung Chang Gung Memorial Hospital were invited to

participate in this study. Infants exhibiting severe congenital abnormalities or having required mechanical ventilation at any time since birth were excluded. The institutional review boards of Keelung Chang Gung Memorial Hospital, Taiwan approved the study project (No. 201901821A3C501), and written informed consent was obtained from the mother of each participant.

Sample and clinical data collection

The mothers were requested to bring the enrolled infants to the Pediatric Clinic of Keelung Chang Gung Memorial Hospital at the time points (1, 6, 12, 24, and 36 months of age) for pathogen detection from nasopharyngeal swabs. Nasopharyngeal swabs were collected with separate cotton-tipped swabs (Copan Swab Applicator, Copan Diagnostics Inc., Brescia, Italy) through the nose and into the nasopharyngeal spaces at the scheduled visits. Initially, one swab per child per visit was taken, transported to the microbiology laboratory within 2 h after collection, and cultured for bacteria using standard methods for identification.[17] Since 2015, two swabs per child were taken if the child was aged 36 months. One sample was collected for bacterial culture, and the other was placed back into the collection tube after sampling and stored at −80 °C for further microbiota analysis. Questionnaire surveys were conducted at each visit to obtain information regarding demographic data, housing and living conditions, socioeconomic status, risk factors for colonization, history of respiratory tract infection, and other clinical parameters. Total IgE was measured (ImmunoCAP, Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden), and a total IgE level ≥100 kU/L was considered to be a sample positive for IgE sensitization.[18]

DNA isolation, 16S ribosomal RNA gene amplification, and DNA sequencing

Bacterial genomic DNA was isolated from nasopharyngeal swabs using a FastDNA Spin Kit (MP Biomedical, Solon, OH, USA) following the manufacturer's instructions. DNA was extracted with 70 μL of DNase/pyrogen-free water, and the purity was quantified by measuring the absorbance at 260 and 280 nm using a spectrophotometer (Nanodrop 1000; Thermo Scientific, Waltham, MA, USA). All samples had an A260-to-A280 absorbance ratio between 1.8 and 2.1. Polymerase chain reaction (PCR) was used to amplify the variable region V3—V4 of the gene encoding 16S rRNA in bacteria using bacteria/archaeal primer 515F/806R with the barcodes.[19] Gel electrophoresis of the PCR products on 2% agarose gels was performed for quality control. Samples with one clear band between 400 and 450 bp were selected for further experiments. Amplicons were purified using the GeneJET Gel Extraction Kit (Thermo Scientific) and quantified using a Qubit dsDNA HS Assay Kit (Qubit) on a Qubit 4.0 fluorometer (Qubit). Sequencing libraries were generated using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB) following the manufacturer's recommendations. Purified libraries were quantified, normalized, pooled, and applied for cluster generation and sequencing on an Illumina HiSeq 2500 platform (Illumina, Inc., San Diego, CA, USA) according to the manufacturer's instructions. The sequence data and mapping file for all the samples included in this study have been deposited in Figshare (https://figshare.com/s/6b545fe1555c345d0f9e).

Sequence processing and data analysis

Amplicons were generated using paired-end 250 bp reads, assembled, and pretreated to obtain clean tags using FLASH.[20] Reads of less than 100 nucleotides and chimeric sequences were detected and removed to obtain effective tags using the UCHIME algorithm.[21] Data analysis was performed using the software ''Quantitative Insights into Microbial Ecology'' (QIIME).[22] Assembled sequences were clustered into operational taxonomic units (OTUs) using Uparse software at 97% sequence identity,[23] and taxonomy classification was assigned on the basis of the full-length 16S rRNA gene database, Greengenes.[24]

Bacterial community profiles were analyzed, and rarefaction curves based on the number of species were generated for each sample from the randomized OTU draws. Microbial community comparisons were made using the Bonferroni correction test, which corrected the *P*-values for multiple comparisons in Unifrac.[25] Abundance differences between groups were tested using the MetaStat method with multiple comparison adjustments.[26] The richness of each sample was calculated using the Chao1 index, and diversity accounting for both relative abundance and evenness was evaluated the Shannon index.[8] Beta diversity was calculated between groups at the OTU genus level, and principal coordinate analysis (PCoA) plot in conjunction with weighted Unifrac and non-metric multidimensional scaling (NMDS) plot in a two-dimensional taxon space based on Bray—Curtis similarities were produced to show clustering between groups.[25,27] Similarity analysis was conducted using the unweighted-pair group method with the arithmetic average (UPGMA) clustering algorithm. Rare OTUs were defined as less than 0.01% of the reads in a given sample, and were removed if they were present in more than 50% of all samples.[28]

Statistical analysis

The baseline clinical characteristics of the three groups were compared using parametric or non-parametric tests. Analysis of variance (ANOVA) was used to analyze the numerical data. If the data were not normally distributed, the Kruskal—Wallis test was used to compare nonparametric data. A chi-square test or Fisher's exact test was used to analyze the categorical data. Statistical significance was set at $p < 0.05$. Data were analyzed using Statistical Package for the Social Sciences (SPSS Statistics for Windows Version 22.0; SPSS Inc., Chicago, IL, USA).

Results

Characterization of the study population

In total, 87 children were enrolled and were further split into three groups, including 36 with allergic respiratory

Table 1 Baseline characteristics of 87 subjects with 3 different phenotypes.

Variables	Grouping			*p*-value
	Allergic respiratory diseases[a] + Atopy[b]	Atopy alone[b]	Controls	
	N = 36	N = 21	N = 30	
Demographics				
Male (%)	20/36 (55.6)	11/21 (52.4)	14/30 (46.7)	0.77
Breastfeeding[c]				
Breastfeeding (%) since birth	26/36 (72.2)	17/21 (81.0)	23/30 (76.7)	0.75
Breastfeeding period (mo) median (interquartile range)	3.0 (0.0–7.0)	9.0 (2.0–18.0)	3.0 (0.0–8.0)	0.18
Exclusive breastfeeding period (mo) median (interquartile range)	0.0 (0.0–6.0)	8.0 (0.0–15.0)	2.0 (0.0–7.3)	0.15
Probiotics usage within 1 mo (%)	11/36 (30.6)	6/21 (28.6)	10/30 (33.3)	0.93
Environmental factors				
No. of the family members median (interquartile range)	4.0 (4.0–6.0)	4.5 (4.0–6.5)	5.0 (4.0–6.0)	0.62
No. of children in the family median (interquartile range)	2.0 (1.0–2.0)	2.0 (1.0–2.0)	2.0 (1.8–2.3)	0.76
Health conditions				
Bacterial colonization[d] (%)	17/36 (47.2)	10/21 (47.6)	15/30 (50.0)	0.97
Preterm birth (%)	6/36 (16.7)	3/21 (14.3)	3/30 (10.0)	0.73
Delivery via NSD (%)	20/36 (55.6)	9/21 (42.9)	21/30 (70.0)	0.15
PICU admission at birth (%)	5/36 (13.9)	4/21 (19.0)	7/30 (23.3)	0.61
Children with Vitamin D deficiency[e] (%)	11/15 (73.3)	4/8 (50.0)	16/23 (69.6)	0.50
Mothers with Vitamin D deficiency[e] (%)	14/17 (82.4)	6/9 (66.7)	16/22 (72.7)	0.64
Total IgE (kU/L) median (interquartile range)	231.0 (111.0–766.8)	229.0 (104.7–290.5)	29.5 (16.4–44.8)	<0.001
URI[f] within 2 weeks (%)	15/36 (41.7)	4/21 (19.0)	4/30 (13.3)	0.14
PCV-13 vaccinated children[g] (%)	28/36 (77.8)	15/21 (71.4)	26/30 (86.7)	0.40
Oral antibiotics usage within 1 mo (%)	3/36 (8.3)	3/21 (14.3)	2/30 (6.7)	0.63

[a] The group with allergic respiratory diseases denotes children with any of the following allergic airway diseases, including asthma or allergic rhinitis.
[b] Atopy is defined as having IgE sensitization (total IgE level ≥100 kU/L); The group with atopy alone denotes children with IgE sensitization without the above allergic respiratory diseases, history of food allergy or atopic dermatitis.
[c] Breastfeeding means that infants had a history of breastfeeding including exclusive breastfeeding for at least 4 weeks.
[d] Bacterial colonization means colonized bacteria identified by the traditional culture method.
[e] Vitamin D deficiency is defined as the level of vitamin D was less than 20 ng/mL.
[f] Upper respiratory tract infection (URI) includes pharyngitis, croup, acute otitis media or acute sinusitis occurring within the recent 2 weeks.
[g] PCV-13 vaccinated children means that children receiving 13-valent pneumococcal conjugate vaccine for a total of ≥3 doses on schedule.
NSD, normal spontaneous delivery; PICU, pediatric intensive care unit; URI, upper respiratory tract infection.

diseases plus atopy, 21 with atopy alone, and 30 healthy controls. Of the 36 cases with allergic respiratory diseases plus atopy (2, asthma alone; 28, allergic rhinitis alone; 6, combined asthma and allergic rhinitis), 2 of them also had other atopic diseases, including atopic dermatitis in 1 and food allergy in 1, respectively. Children with non-atopy allergic respiratory diseases were not included in this study because the number of children with non-atopy allergic respiratory diseases (N = 6) was relatively small. All the children were at the age of 36 months, and no specific clinical diseases (i.e., hospitalization, pneumonia or other severe bacterial infections requiring parenteral antibiotics therapy) occurred to them within one month before the collection of nasopharyngeal swabs. Three factors, including demographic features, environmental characteristics, and health conditions, were compared among the three groups (Table 1). Except for total IgE levels being significantly higher in children with allergic respiratory diseases plus atopy or those with atopy alone ($P < 0.001$), no other characteristics were significantly different between these three groups.

Nasopharyngeal bacterial community composition and abundance

A range of reads from 55,659 to 110,182 was obtained per sample, and a total of 1700 OTUs were detected in all subjects. Rarefaction curves showed that a plateau of species richness was achieved in approximately 18,000 reads per sample (Supplementary Fig. S1). To control sample heterogeneity, 18,000 random reads were used as the minimum sampling depth to capture diversity. The bacterial composition and abundance at the phylum and

genus levels are shown in Fig. 1.Taxonomic classification revealed a high prevalence of members of the phylum Proteobacteria (45.7% of the total number of sequences obtained), followed by those of the phyla Firmicutes (29.3%), Actinobacteria (15.3%), Bacteroidetes (4.4%), and others (Fig. 1A). *Pseudomonas* (22.3%), *Corynebacterium* (12.7%), *Paenibacillus* (11.8%), *Streptococcus* (3.6%), and *Brevundimonas* (3.2%) were the top five predominant genera (Fig. 1B).

Differential analysis of the abundance of microorganisms for airway allergies

At the phylum level, Chloroflexi, Patescibacteria, Tenericutes, and Nitrospirae were more frequently found in children with allergic respiratory diseases plus atopy than in healthy controls (Fig. 2A); Deinococcus_Thermus, Elusimicrobia, Rokubacteria, Armatimonadetes, and Dependentiae were more abundant in healthy controls than in children with atopy (Fig. 2B). The comparison of the abundance of members of genera among the three groups (allergic respiratory diseases plus atopy, atopy alone, and healthy controls) are shown in Table 2. Members of the genera *Bacillus* (Firmicutes), *Ruminococcaceae* (Firmicutes), *Rhodococcus* (Actinobacteria), *Acinetobacter* (Proteobacteria), *Moraxella* (Proteobacteria), and *Asaia* (Proteobacteria) were significantly more abundant in children with allergic respiratory diseases (or atopy) than in healthy controls; however, the members of the genera *Enterococcus* (Firmicutes), *Alkanindiges* (Proteobacteria), *Rickettsia* (Proteobacteria), and *Rhizobacter* (Proteobacteria) were predominant in healthy controls than in children with allergies. Furthermore, bacteria in genera between children with atopic respiratory diseases plus atopy and atopy alone were also compared. The result

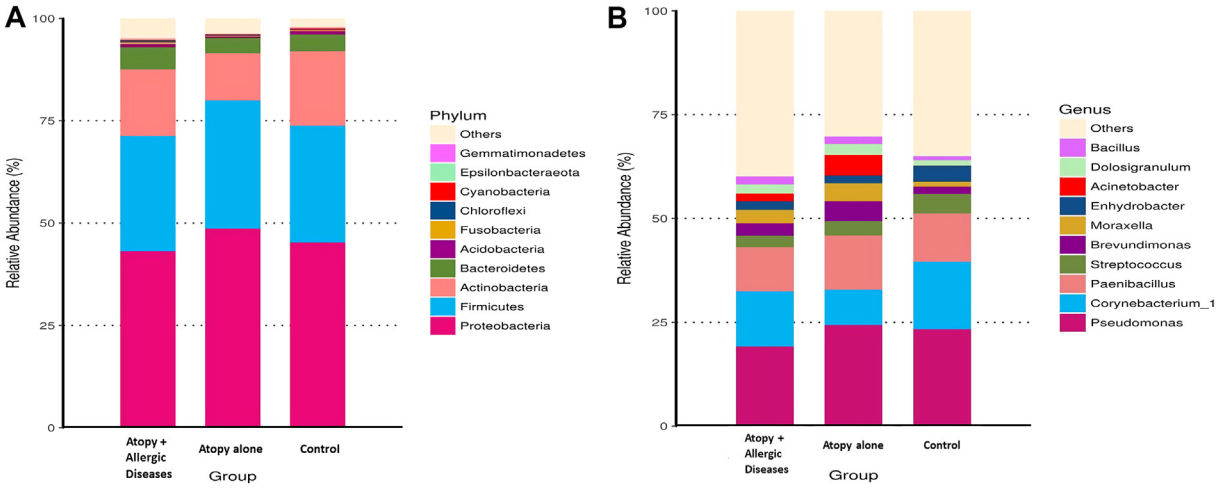

Figure 1. Nasopharyngeal microbial composition and abundance at the phylum and genus level **(A)** Bacterial composition and abundance at the phylum level. Each bar represents the top 10 bacterial species ranked by the relative abundance in children with allergic respiratory diseases plus atopy, atopy alone, and healthy controls. **(B)** Bacterial composition and abundance at the genus level. Each bar represents the distribution of the ten most abundant genera in children with allergic respiratory diseases plus atopy, atopy alone, and healthy controls.

Figure 2. Comparison of nasopharyngeal microbial composition and abundance between children with allergic respiratory diseases plus atopy, atopy alone, and healthy controls **(A)** UPGMA clustering trees based on Welch's *t*-test bar plot of bacterial taxon phyla between children with allergic respiratory diseases plus atopy and healthy controls. **(B)** UPGMA clustering trees based on Welch's *t*-test bar plot of bacterial taxon phyla between children with atopy alone and healthy controls.

Table 2 Differences of bacteria in phyla and genera between children with atopic respiratory diseases + atopy, atopy alone and healthy control.

Phylum/Genus	Atopic respiratory diseases + atopy (N = 36)	Atopy alone (N = 21)	Healthy control (N = 30)	Atopic respiratory diseases + atopy *vs.* atopic alone	Atopic respiratory diseases + atopy *vs.* control	Atopic alone *vs.* control
	mean ± SD (%)	mean ± SD (%)	mean ± SD (%)	*P*-value	*P*-value	*P*-value
Firmicutes/*Enterococcus*	0.03 ± 0.03	0.34 ± 1.46	1.91 ± 4.63	0.010	<0.001	0.022
Firmicutes/*Bacillus*	1.98 ± 2.46	1.83 ± 1.78	0.98 ± 0.93	0.596	0.025	0.025
Firmicutes/*Ruminococcaceae*	0.53 ± 0.59	0.54 ± 0.95	0.29 ± 0.34	0.158	0.032	0.006
Actinobacteria/*Rhodococcus*	0.53 ± 0.89	1.16 ± 4.53	0.09 ± 0.16	0.029	<0.001	0.020
Proteobacteria/*Acinetobacter*	1.81 ± 3.91	4.98 ± 12.39	0.07 ± 0.06	0.013	<0.001	<0.001
Proteobacteria/*Moraxella*	3.08 ± 4.74	4.24 ± 5.96	1.21 ± 1.86	0.233	0.006	<0.001
Proteobacteria/*Alkanindiges*	0.06 ± 0.22	0.08 ± 0.23	0.36 ± 0.68	0.462	<0.001	0.003
Proteobacteria/*Rickettsia*	0.05 ± 0.16	0.00 ± 0.01	0.23 ± 0.34	0.021	<0.001	<0.001
Proteobacteria/*Rhizobacter*	0.18 ± 1.04	0.00 ± 0.00	3.28 ± 8.52	0.127	0.003	0.013
Proteobacteria/*Asaia*	0.20 ± 0.70	0.16 ± 0.33	0.00 ± 0.01	0.646	0.013	<0.001

The data "mean ± SD" means the relative abundance of bacteria. The percent of total numbers of sequences are shown for each split level. Only taxonomic classification with more than 100 sequences or 0.01% of total sequences, and statistically significant difference are shown.

showed that *Enterococcus*, *Rhodococcus* and *Acinetobacter* were less abundant; *Rickettsia* was more predominant in children with atopic diseases plus atopy than those with atopy alone. No differences in the genera *Bacillus*, *Ruminococcaceae*, *Moraxella*, *Asaia*, *Alkanindiges*, and *Rhizobacter* were found among the two groups. All these differences were statistically significant.

Bacterial richness and diversity categorized by atopic diseases

The Chao1 and Shannon indices, which were measured for species richness and diversity, respectively, were calculated and analyzed (Fig. 3). Children with atopy alone had a significantly lower Chao1 index than healthy controls ($P = 0.035$). In addition, a relatively higher Shannon index was present in children with allergic respiratory diseases plus atopy or atopy alone than in the healthy controls; however, the difference was not statistically significant. The statistics of beta diversity using the PCoA and NMDS showed that no significant difference was found in the microbial community cluster patterns concerning airway allergies (Supplementary Fig. S2).

Association between total IgE levels and the microbiome

Correlations between serum total IgE levels and the genera of identified bacteria among children with different allergic phenotypes are shown in Fig. 4. *Acinetobacter* spp., *Moraxella* spp., *Asaia* spp., and *Rhodococcus* spp. were significantly positively correlated with total IgE levels ($P < 0.01$),

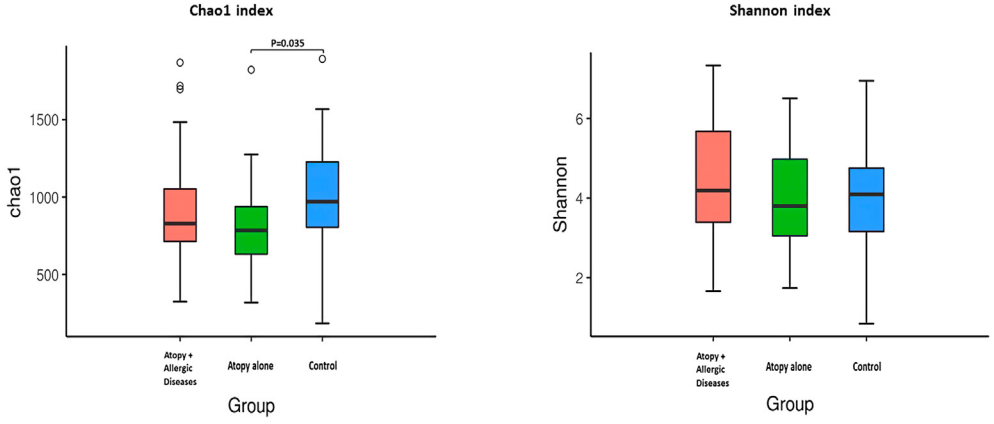

Figure 3. Differences and comparisons of species richness and diversity among children with allergic respiratory diseases plus atopy, atopy alone, and healthy controls. Species richness calculated as the Chao1 index. Species diversity calculated as the Shannon index.

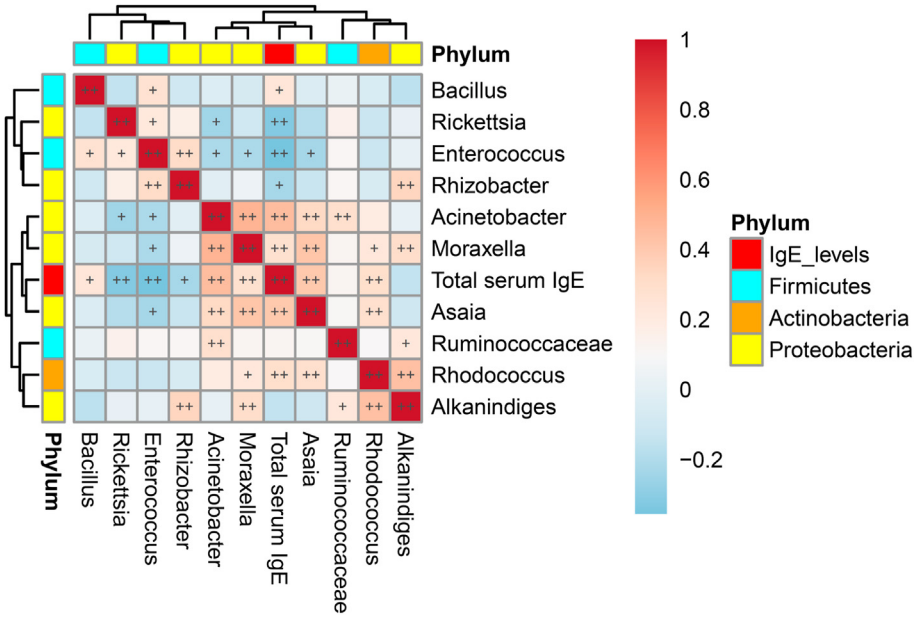

Figure 4. Heatmap of correlations of identified microbiomes from different allergic phenotypic children with total IgE levels. Color intensity represents the magnitude of correlation. Red color represents positive correlations; blue color represents negative correlations. + symbol means a *P*-value < 0.05; ++ symbol means a *P*-value < 0.01.

whereas the genera *Enterococcus*, and *Rickettsia* were found to be negatively correlated with total IgE levels ($P < 0.01$).

Discussion

With the application of an 16S rRNA-molecular based sequencing method, our study showed differences in the nasopharyngeal microbiome composition among children with three distinct phenotypes of respiratory allergies, indicating that changes in bacterial communities may contribute to the development of allergic respiratory diseases. In fact, the link between bacterial colonization and asthma has been recognized for some time. A study with a traditional culture-based identification method demonstrated that neonates whose hypopharynx were colonized with *Streptococcus pneumoniae*, *Hemophilus influenzae*, or *Moraxella catarrhalis* were at increased risk for recurrent wheezing and asthma in childhood.[29] However, the role of these bacteria in the pathogenesis of asthma remains unclear.

Allergic asthma is a Th2-dominant inflammatory process of the lower airways, which is characterized by the overexpression of pro-inflammatory cytokines interleukin 4 (IL-4), IL-5, and IL-13, and can be initiated through the activation of the immune system by environmental bacterial exposure.[6] The lower airway microbiome resembles that of the upper airway, such as the nasopharynx, where microbes may gain entry through inhalation of the surrounding environment or microaspiration.[30] Environmental bacterial exposure may influence the composition of the lower airway mucosal microbiota and modulate host immunity, leading to increased lung inflammation, immune dysfunction, and asthma development.[30]

The airway microbiome composition is known to be highly correlated with the development of childhood asthma, thus raising the possibility that dysbiosis of specific bacterial members within the airway may potentiate the respiratory allergic response.[30] In previous studies of cohorts of patients with asthma, five major phyla were found to be obtained from the lower airways: Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, and Fusobacterium. Members of the phylum Proteobacteria, including the genera *Moraxella*, *Hemophilus*, and *Neisseria*, were detected to be dominant in the airways of asthmatic patients.[30,31] In our study, as shown in Table 2, two major phyla, namely Proteobacteria and Firmicutes, were noted in children with allergies, with the dominance of Proteobacteria with *Moraxella* observed in the nasopharynx of children with airway allergies, which was consistent with a previous study. The detailed mechanism of the process still needs to be elucidated, but *in vitro* testing with *Moraxella* spp. revealed that this bacterium can induce pulmonary epithelial damage and inflammatory cytokine expression (IL-8, IL-13, and IL-17) and thus, lead to the development of asthma.[32] Additionally, several reports have revealed that the genus *Acinetobacter* is less abundant, and that the genera *Alkanindiges* and *Rickettsia* are more abundant in adults with airway allergies.[6] In our study, *Acinetobacter* appeared to more abundant, whereas *Alkanindiges* and *Rickettsia* were less commonly seen in children with allergic airway diseases/atopy alone than those with healthy controls. However, if we only compared the relative abundance of bacterial genera between children with atopic respiratory diseases plus atopy and atopy alone, *Acinetobacter* was found to be less abundant and *Rickettsia* to be more abundant in those with atopic respiratory diseases plus atopy, compared with those with atopy alone.

This finding is consistent with the previous study as mentioned above ,[6] which means that certain bacterial genera (i.e., *Acinetobacter* and *Rickettsia*) may play an important role in cases with allergic respiratory tract diseases, especially for those with allergic constitutions.

In our study, we found an inverse association between bacterial richness and allergies. As mentioned above, the initiation of asthma could be through the activation of the immune system by components of the bacterial walls or bacterial products (e.g., endotoxin) within the lower airways.[30] Several studies have shown that early life exposure to bacterial endotoxins may protect against later atopy. Furthermore, high levels of bacterial endotoxin may reduce sensitization and atopic asthma.[30,33] This potentially protective effect is consistent with the "hygiene hypothesis," which postulates that children who grow up in a microbially more hygienic environment (i.e., environment with less bacterial richness) may be at increased risk of developing respiratory allergies. In addition, the analysis of beta diversity showed that there was no significant difference of microbial community among the three groups. Beta diversity means that the whole species diversity, not the comparison of certain specific bacterial phyla/genera among the three groups. Thus, as the reports of previous studies,[8,34] the significant difference of the relative abundance of *Acinetobacter* and *Rickettsia* was found between the three groups, but no difference of beta diversity was noted among them in our study.

Serum total IgE is secreted by B-cells that are stimulated by inhaled allergens, with a high IgE level considered a risk for developing allergic diseases.[35] In our study, the genera *Acinetobacter*, *Moraxella*, and *Asaia* of the phylum Proteobacteria, as well as the genus *Rhodococcus* of the phylum Actinobacteria, were positively correlated with serum total IgE levels, and appeared to be risk factors for airway allergies. In addition, the genus *Ruminococcaceae* of the phylum Firmicute was also relatively abundant in children with allergies; however, it did not correlate with serum total IgE levels. The possible cause of this result may be that certain bacteria belonging to Firmicutes may modulate the immune function of T cells by presenting antigens contributing to respiratory allergies rather than eliciting adaptive immune responses by modulating B-cell antibody production.[34] In contrast, the genera *Enterococcus*, *Rickettsia*, and *Rhizobacter* were inversely correlated with total IgE levels and appeared to be negatively associated with airway allergies. Thus, the abundance of the genera *Enterococcus*, *Rickettsia*, and *Rhizobacter* in airways may lead to the prevention of airway allergies by decreasing B-cell IgE production.

A major limitation of this study is that the number of enrolled cases was relatively small; thus, it might have decreased the statistical power of the results. A strength of this study, however, is that sample collection and clinical evaluation were performed by the same well-trained research assistants and experienced physicians, respectively, leading to the study result being potentially accurate and consistent.

In conclusion, alterations in the composition of the nasopharyngeal microbiota may influence the prevalence of childhood respiratory allergies. An inverse association was found between bacterial richness and allergies, suggesting that children living in a microbially hygienic environment may be at increased risk of developing respiratory allergies. A correlation between serum IgE levels and abundance of *Acinetobacter* spp., *Moraxella* spp., *Asaia* spp., and *Rhodococcus* spp. related to airway allergies suggests that the modulation of specific subsets of airway microbial dysbiosis may potentially contribute to children's susceptibility to allergic respiratory diseases by increasing (or decreasing) B-cell IgE production. However, some specific genera [i.e., *Ruminococcaceae* (Firmicutes)] may lead to the initiation of airway allergies by modulating the immune function of T-cell. Further studies are needed to investigate the association between specific microbiomes and allergic respiratory diseases by performing related functional analyses in the future.

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Declaration of competing interest

None.

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Appendix A. Supplementary data

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