

ORIGINAL ARTICLE

Rhinitis, Sinusitis, and Upper Airway Disease

Different upper airway microbiome and their functional genes associated with asthma in young adults and elderly individuals

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Abstract

Background: Microbes in the airway have been shown to be associated with the pathogenesis of asthma. The upper airway microbiome influences the dysbiosis of the lower airway microbiome. However, to date, the influence of upper airway microbiome for adult and elderly asthma has not been fully elucidated. Here, the metagenome of upper airway microbiome of young adults and elderly was analyzed to identify their association with adult asthma.

Methods: Nasopharyngeal swabs were collected from young adult and elderly asthma patients and non-asthmatic subjects. The compositions and functional genes of airway microbiome were analyzed by high-throughput sequencing.

Results: The composition of microbiota differed between young adult and elderly, and it was different between asthmatics and non-asthmatics in each age group. Different bacteria were related to FEV₁% predicted in each age group. Genes related to lysine degradation, N-glycan biosynthesis, caprolactam degradation, and PPAR signaling pathway, which could be related to the reduction in inflammation and degradation of air pollutants, were higher in non-asthmatics. Genes related to pentose phosphate pathway, lipopolysaccharide biosynthesis, flagella assembly, and bacterial chemotaxis—which may all be related to increased inflammation and colonization of pathogenic bacteria—were higher in young adult asthmatic patients. However, the functional genes of airway microbiome in elderly patients were not significantly different according to asthma morbidity.

Conclusions: These results suggest that the composition and function of upper airway microbiome could influence asthma pathogenesis, and the microbiome could play various roles depending on the age group.

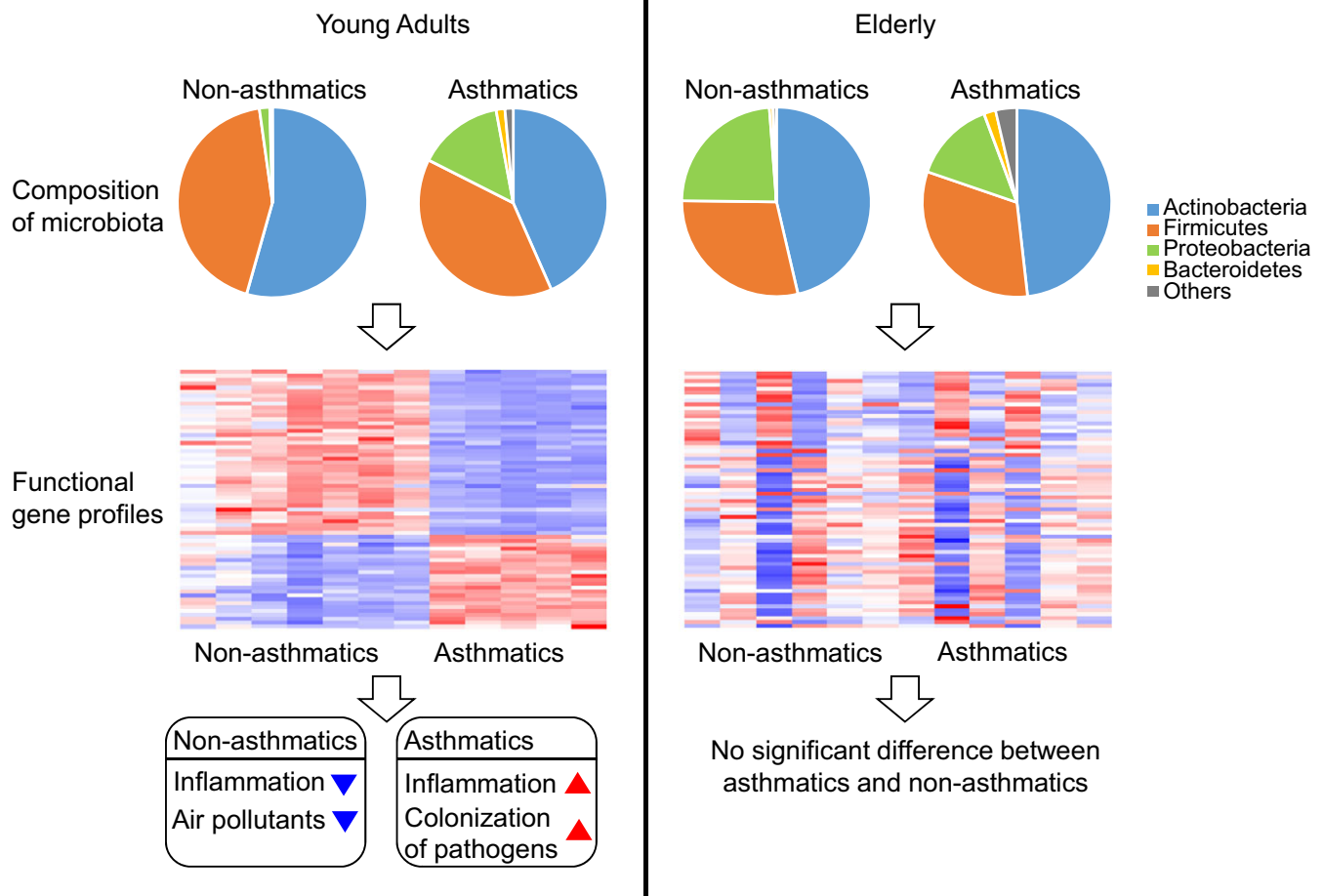
KEYWORDS

adult, asthma, elderly, microbiome, upper airway

1 | INTRODUCTION

Asthma is a chronic disorder characterized by reversible airway obstruction, chronic airway inflammation, and airway

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GRAPHICAL ABSTRACT

The upper airway microbiome was different between young adults and elderly, and their association with asthma was also different. The microbiome genes reducing airway inflammation and degrading air pollutants were lower in asthmatics, whereas genes enhancing inflammation and mucosal bacterial colonization were higher in asthmatics of young adults. The composition and function of upper airway microbiome could influence asthma pathogenesis, and the microbiome could play various roles depending on the age group.

hyperresponsiveness.^{1,2} Airway inflammation plays a central role in asthma pathogenesis; however, the mechanism of this process has not been clearly elucidated to date. The occurrence of asthma is a complex process involving the interplay of various host and environmental factors. The host immune status, genetic factors, and mucosal barrier conditions may determine the susceptibility and development of asthma. Moreover, a variety of environmental factors, including allergens, respiratory infections, smoking, air pollutions, occupational exposures, and diets, has been shown to be involved.² Recently, microbiota in the airway has been studied due to their potential role in the pathogenesis of asthma.

Various microbes have colonized the airway, showing its association with the development and severity of asthma.³ The resident microbes in the airway can regulate the host immunocompetence in the lung with an infection and can be a reservoir of respiratory pathogens with antibiotic-resistant or virulence genes.^{4–6} Several studies have reported that the composition of the airway microbiome in asthma patients is altered when compared with healthy controls.^{7–10}

The relative abundance of Proteobacteria and bacterial diversity were higher in asthmatics compared with normal controls. Human microbiome is influenced by various factors, including the central physiological processes such as aging. Therefore, the airway microbiome related to asthma in elderly patients could be different to that in the younger adult patients. However, there is currently limited information regarding the airway microbiome of elderly asthmatics. Furthermore, clinical characteristics and risk factors of asthma are different between young adults and older adults, including elderly. In children and young adults, atopy is the most important risk factor for asthma. However, non-atopic asthma is prevalent in the elderly. Instead of atopy, other risk factors such as smoking, chronic rhinosinusitis, staphylococcal enterotoxin sensitization, and sarcopenic obesity are indicated for risk factors in elderly patients.¹¹ Different clinical characteristics between asthma in young adult and elderly patients suggest that they might have different associations with airway microbiome.

The upper airway is continuously exposed to the outside world and is the point of entry to the lower airway. The alteration of upper airway

microbiome can cause dysbiosis of lower airway microbiome, resulting in lower airway diseases. To the best of our knowledge, only a few studies have addressed the possible etiologic influence of the upper airway microbiome on the development of lower airway diseases in adults. The upper airway microbiome of HIV (human immunodeficiency virus)-infected patients, pandemic H1N1 influenza virus-infected patients, and elderly pneumonia patients were analyzed.^{12–14} However, studies on the upper airway microbiome in chronic respiratory diseases, such as asthma, in adult and elderly patients have been limited.

The first objective of the present study was to compare the composition and functional genes of upper airway microbiome between young adults and the elderly. The second objective was to identify the differences in upper airway microbiome between non-asthmatics and asthmatics in each age group. The results indicate that the upper airway microbiome is different between young adult and elderly patients even within the same asthmatic status and they can interact differently with the host.

2 | METHODS

2.1 | Study subjects and sample collection

Eighty subjects (30 asthmatic and 10 non-asthmatic young adults between the ages of 18 and 45 years; 30 asthmatic and 10 non-asthmatic elderly patients over the age of 65 years) were enrolled. Patients with asthma were recruited with the following inclusion criteria: (a) airway obstruction reversibility documented by an increase in forced expiratory volume in one second (FEV₁) of 12% and 200 mL after inhalation of 200 µg of salbutamol, or 4 weeks of anti-inflammatory treatment according to Global Initiative for Asthma (GINA) guideline, or airway hyperresponsiveness documented by a methacholine provocation concentration causing a 20% fall in FEV₁ (PC20) of ≤16 mg/mL, in the past or at the time of enrollment; and (b) presence of persistent characteristic asthma symptoms and

physician's diagnosis of asthma. The severity of asthma was assessed retrospectively from the level of treatment to control symptoms and exacerbations.¹⁵ Details regarding patient enrollment and collection of specimens are provided in the supplementary document.

2.2 | DNA extraction and pyrosequencing

Metagenomic DNA was extracted from nasopharyngeal swabs using RNeasy PowerMicrobiome Kit (Qiagen, Valencia, CA, USA). The 16S rRNA gene was amplified and used for pyrosequencing library.¹⁶ Details on the preparation process are provided in the supplementary document.

2.3 | Whole metagenome sequencing

A total of 24 samples from non-smokers were randomly selected from each group (6 non-asthmatic and 6 asthmatic young adults; 6 non-asthmatic and 6 asthmatic elderly patients), for whole metagenome sequencing. Details on sequencing are provided in the supplementary document.

2.4 | Analyses of microbiota and whole metagenome sequence

To analyze the composition of microbiota, sequences obtained from pyrosequencing were analyzed as described previously.^{16,17} For analyses of whole metagenome sequences, sequences obtained from the HiSeq 2500 were analyzed as described previously.^{17,18} Details on analyses are provided in the supplementary document.

2.5 | Statistical analysis

The differences between bacterial taxa among the groups were tested by Kruskal-Wallis rank-sum test in R software (ver. 3.2.2).

TABLE 1 Clinical characteristics of analyzed subjects

Group	Adults			Elderly		
	Non-asthma	Asthma	P value	Non-asthma	Asthma	P value
Subjects (n)	10	30		10	30	
Age (years)	25.4 ± 6.2	34.1 ± 7.0	0.002	67.3 ± 3.5	72.5 ± 5.4	0.004
Male (%)	50	36.7	0.473	20	44.8	0.176
BMI (kg/m ²)	21.4 ± 2.4	22.7 ± 3.8	0.212	25.0 ± 3.5	24.3 ± 3.4	0.724
Smoking (%)	20	26.7	0.693	10	24.2	0.358
FVC (% pred)	87.2 ± 6.7	90.4 ± 11.9	0.189	103.1 ± 12.6	92.3 ± 15.0	0.076
FEV1 (% pred)	94.4 ± 10.2	91.4 ± 15.2	0.684	115.5 ± 14.9	90.6 ± 20.4	0.004
FEV1/FVC (% pred)	80.2 ± 0.8	78.0 ± 1.6	0.177	72.0 ± 1.6	70.8 ± 1.3	0.035
Total IgE (IU/mL)	NA	372.6 ± 666.3		NA	107.3 ± 91.4	
Blood eosinophils (×10 ³ cell/uL)	NA	0.55 ± 0.48		NA	0.26 ± 0.25	
Family history (%)	50	66.7	0.362	10	41.4	0.076
Chronic disease (%)	20	30	0.558	70	72.4	0.902
Infectious disease within 3 months (%)	0	13.3	0.241	0	6.9	0.424

Kruskal-Wallis rank-sum test was used to compare the frequency of each taxon among the groups, and *P* values were calculated using the approximate chi-squared distribution. Mann-Whitney *U* test was used to identify statistically significant pairwise differences between the groups. Significantly different KO categories in the functional profile results were identified using Kruskal-Wallis rank-sum test in R. Corrections were made using the Benjamini-Hochberg false discovery rate multiple testing correction. The correlation between clinical measurements and relative abundance of specific microbes was analyzed using Spearman's rank correlation in SPSS (ver. 22).

3 | RESULTS

3.1 | Characteristics of the study subjects

In this study, we used specimens collected from 30 asthmatic and 10 non-asthmatic young adults, as well as 30 asthmatic and 10 non-asthmatic elderly patients. The characteristics of these subjects are summarized in Table 1.

In the demographic characteristics, although there was a difference in age between asthmatics and non-asthmatics in both age groups, gender was not significantly different between the two groups. Among elderly individuals, FEV_{1%} predicted at the time of airway microbiome sample collection was lower in asthmatics (90.6 ± 20.4) than in non-asthmatics (115.5 ± 14.9) (*P* < 0.01). Moreover, the ratio of FEV₁/force vital capacity (FVC) was also significantly lower in asthmatics (70.8 ± 1.3) than in non-asthmatics (72.0 ± 1.6) among elderly individuals (*P* < 0.05). Although FEV₁ and the ratio of FEV₁/FVC were lower in asthmatics than in non-asthmatics among young adults, the differences were not statistically significant. Other characteristics of BMI, smoking, FVC, family history, and comorbidities were not significantly different between asthmatics and non-asthmatics in both age groups. The total serum IgE levels and blood eosinophil counts were significantly higher in young adult asthmatics (IgE: 372.6 ± 666.3 IU/mL and eosinophil: 0.55 ± 0.48 10³ cells/uL) than in elderly asthmatics (IgE: 107.3 ± 91.4 and eosinophil: 0.26 ± 0.25; *P* < 0.05) (Table 1).

3.2 | Comparison of upper airway microbiota between young adults and elderly

A total of 1 107 994 reads (529 153 reads for young adults; 578 841 reads for elderly; average sequence length, 466.5 bp) were obtained from 79 samples. The calculated diversity indices after normalization are summarized in Table S1. There were no significant differences between young adults and elderly individuals with respect to the diversity indices (*P* > 0.05). However, the composition of airway microbiota was different between the two groups in both non-asthmatics and asthmatics (Figure S1). In non-asthmatics, the proportion of Proteobacteria was significantly higher in elderly individuals than in young adults (*P* < 0.05). The relative abundances of *Propionibacterium* and uncultured *Corynebacteriales* were significantly higher

in young adults than in elderly individuals (*P* < 0.05). In asthmatics, the composition of phylum was not significantly different between the two groups. However, the proportions of *Staphylococcus*, *Propionibacterium*, and *Moraxella* were higher in young adults than in elderly individuals (*P* < 0.05).

The difference of age between non-asthmatics and asthmatics was significant in both groups (Table 1). Thus, the differences in diversity and microbiota by age were compared within each group (Figure S2). There were no significant differences in diversity and composition of microbiota between the groups according to age. Therefore, we compared the airway microbiome between asthmatics and non-asthmatics in each group.

3.3 | Comparison of airway microbiota between asthmatics and non-asthmatics in young adults and elderly

The airway microbiota was compared between asthmatics and non-asthmatics among young adults (Figure 1). The diversity was not significantly different between asthmatics and non-asthmatics, and the microbiota was not separated in the PCoA plot (*P* > 0.05; Figure 1A and B). Four phyla of Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes were dominant in the upper airway microbiota of young adults. The relative abundances of Proteobacteria (average 14.4%) were significantly higher in asthmatics than (1.7%) in non-asthmatics (Figure 1C; *P* < 0.05). Although the average proportions of the other phyla were different between asthmatics and non-asthmatics, there was no statistically significant difference between the groups. Frequently detected genera (average proportion >1% of all microbiota and detected in 50% of samples) were compared between the groups (Figure 1D). The relative abundance of uncultured *Corynebacteriales* was significantly higher in non-asthmatics (2.4%) than that in asthmatics (1.7%; *P* < 0.01).

Among the elderly, the diversity was not significantly different between asthmatics and non-asthmatics, and the microbiota was not separated by the presence or absence of asthma in the PCoA plot (*P* > 0.05; Figure S3). The dominant phyla in the upper airway microbiota of elderly individuals were the same as those of young adults. In contrast to young adults, the relative abundances of phyla were not significantly different between asthmatics and non-asthmatics among the elderly. However, the relative abundance of *Moraxella* was higher in non-asthmatics (average 19.9%) than in asthmatics (1.3%) among the elderly (*P* < 0.05).

3.4 | The associated microbiota members with lung function

The significant associations between microbes and clinical features were analyzed by Spearman's correlation (Figure 2). The relative abundances of *Prevotella*, *Neisseria*, and *Fusobacterium* were negatively correlated with FEV₁ predicted (%) in the young adult group. For the elderly group, the relative abundances of *Burkholderia* and *Psychrobacter* were positively correlated with FEV₁ predicted (%).

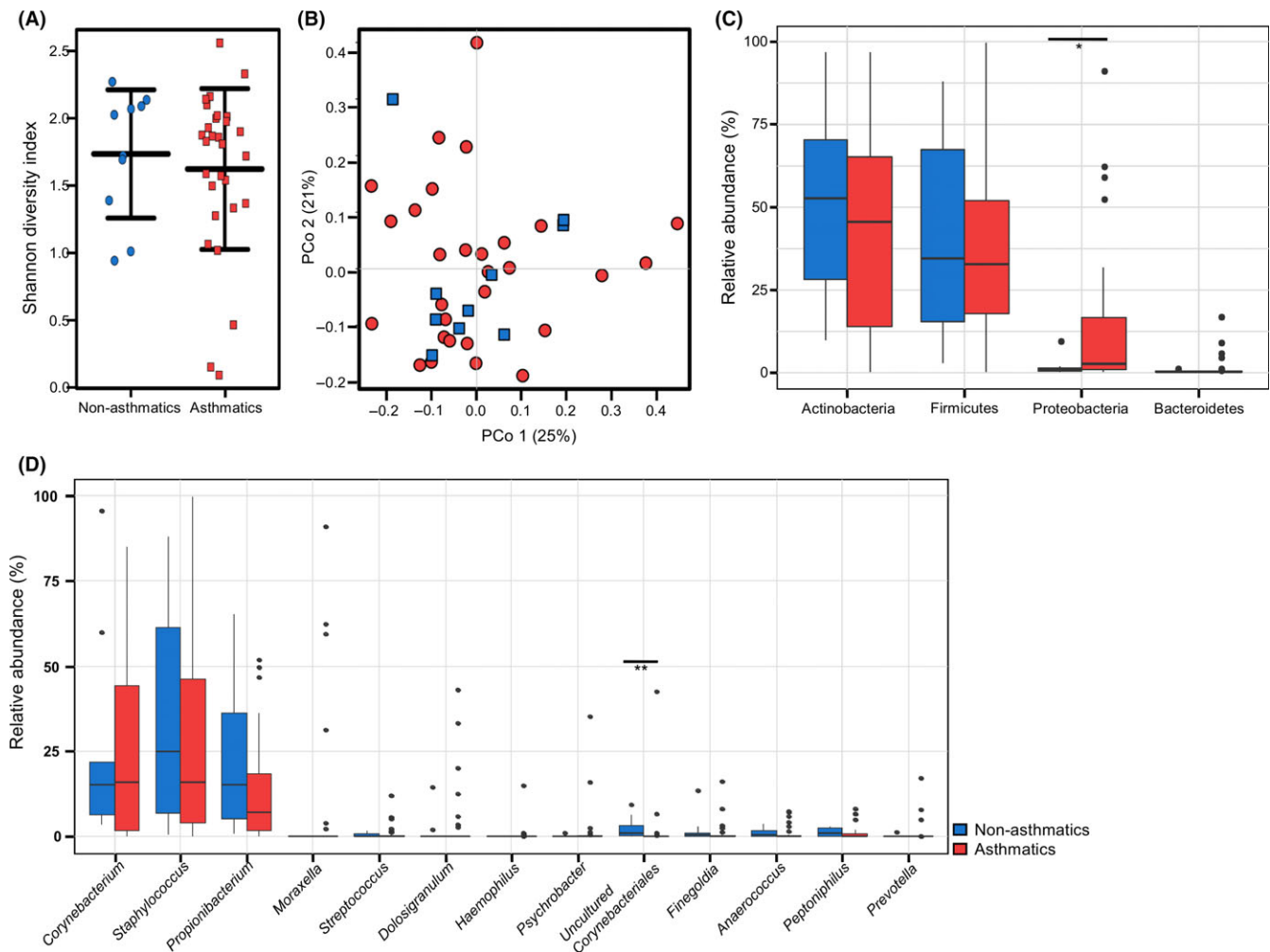


FIGURE 1 Comparison of diversity and composition of airway microbiota from young adults. (A) Shannon diversity index was compared between non-asthmatic subjects and asthmatic patients after normalization of the read number for each sample. (B) The microbiota was compared using the PCoA plot based on UniFrac distance. (C) The composition of phylum was compared between the groups. (D) Genus composition was compared between the groups. Frequently detected genera (average proportion >1% of all microbiota and detected in 50% of adults samples) were selected for comparison. The significance of differences between the groups was tested using Mann-Whitney test. (* $P < 0.05$; ** $P < 0.01$) [Colour figure can be viewed at wileyonlinelibrary.com]

Contrastingly, the relative abundance of *Corynebacterium* was negatively correlated with FEV₁ predicted (%).

3.5 | Different functional genes of airway microbiome between asthmatics and non-asthmatics

The significantly different pathways (corrected $P < 0.05$) were compared between asthmatics and non-asthmatics among the young adults via heat map analysis (Figure 3). The pathways in general information pathway, including translation, folding, sorting, and degradation, were higher in non-asthmatics than in asthmatics (Group 1 pathways). The metabolic pathways in carbohydrate metabolism, metabolism of cofactors and vitamins, biosynthesis of other secondary metabolites, and antimicrobial resistance were higher in asthmatics than in non-asthmatics (Group 2 pathways). A patient with asthma (#9) and a non-asthmatic control (#1) had different gene abundances to their groups. In contrast, there were no significant

differences between asthmatics and non-asthmatics among the elderly group (corrected $P > 0.05$; Figure S4).

Among Group 1 pathways, comparisons of the frequently detected pathways (over 60% of analyzed subjects with identifying contributed species) and contributed bacteria to metabolic pathway between groups are presented in Figure 4. The relative abundances of lysine degradation, N-glycan biosynthesis, caprolactam degradation, and PPAR signaling pathway were significantly lower in asthmatics than in non-asthmatics. Different members of airway microbiota were related to these pathways between non-asthmatics and asthmatics. *Corynebacterium accolens* and *Propionibacterium acnes* were mainly contributed to the higher abundances of these pathways in non-asthmatics. However, *Bacteroides caccae*, *Escherichia coli*, *Veillonella parvula*, and *Bifidobacterium longum* were mainly related to these pathways in asthmatics. The other KO categories with higher abundance in non-asthmatics are summarized in Table S2.

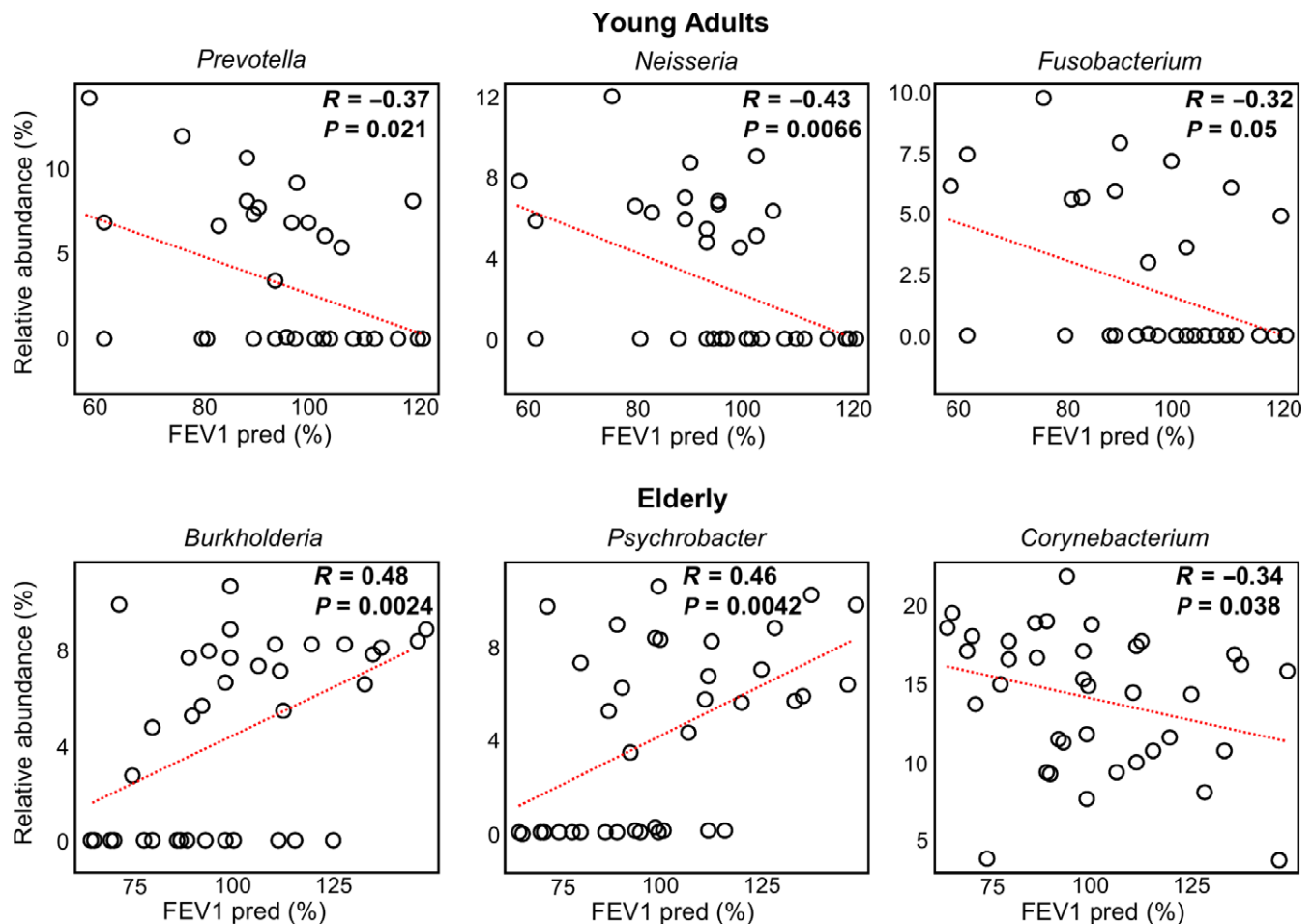


FIGURE 2 Correlation of bacterial genera with clinical features. In the young adult group, *Prevotella*, *Neisseria*, and *Fusobacterium* were related to FEV₁ prediction %. In the elderly group, *Burkholderia*, *Psychrobacter*, and *Corynebacterium* were related to FEV₁ prediction %. The association was analyzed using Spearman's rank correlation test [Colour figure can be viewed at wileyonlinelibrary.com]

Four frequently detected metabolisms in Group 2 pathways and their related species were compared between asthmatics and non-asthmatics (Figure 5). The relative abundances of pentose phosphate pathway, lipopolysaccharide biosynthesis, flagellar assembly, and bacterial chemotaxis were significantly higher in asthmatics than in non-asthmatics. *C. accolens*, *P. acne*, and *Staphylococcus aureus* were mainly contributed to these pathways in non-asthmatics. Contrastingly, *B. caccae*, *E. coli*, and *V. parvula* were mainly related to these pathways in asthmatics. The other KO categories with higher abundance in asthmatics are summarized in Table S3.

4 | DISCUSSION

In this study, the composition and functional profiles of upper airway microbiota were analyzed between asthmatics and non-asthmatics among young adults and elderly individuals. The airway microbiota was different between young adults and elderly individuals, and different microbes were related to the lung function. The functional genes of upper airway microbiome were significantly different

between asthmatics and non-asthmatics only in young adults. The microbiome genes in non-asthmatics could be related to reducing airway inflammation and degrading air pollutants through lysine degradation and caprolactam degradation,^{19,20} whereas those in asthmatics could be related to increasing inflammation and colonizing pathogenic bacteria in the airway through pentose phosphate pathway and bacterial chemotaxis pathway.^{21,22} These differences were caused by different microbial members between asthmatics and non-asthmatics. However, the functional gene profiles of elderly individuals were not significantly different between the groups in this study.

The different composition of upper airway microbiota between young adults and elderly individuals was observed in the present study. Although Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes were the dominant phyla in both age groups, the relative abundances were significantly different between young adults and elderly individuals even within the asthmatic patients (Figure S1). The higher abundance of Proteobacteria in young adult asthmatics was consistent with the findings from most previous studies (Figure 1).^{8,23,24} However, the specific genera and species identified in

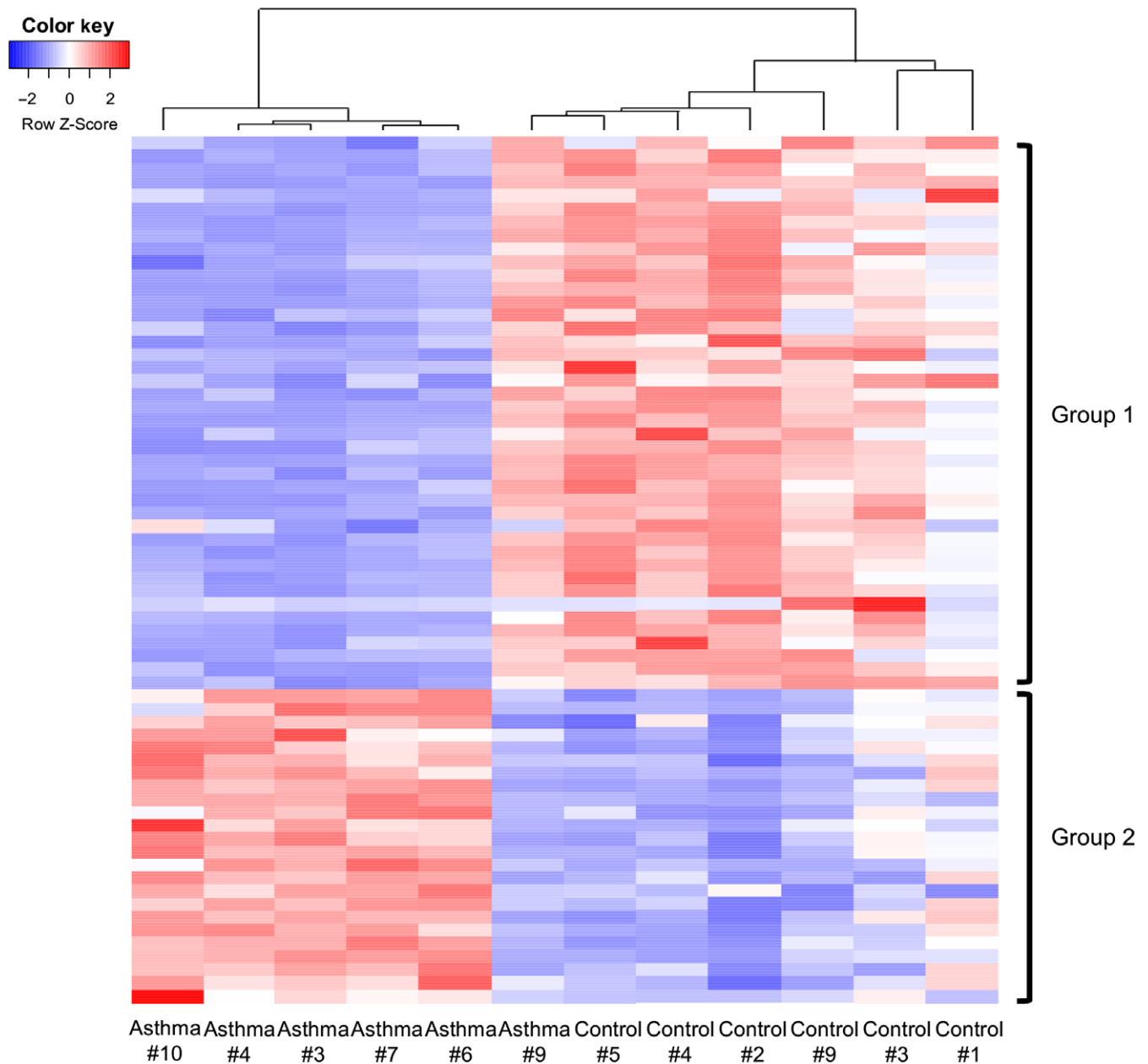


FIGURE 3 Differently detected pathways of airway microbiome in young adults were clustered in Heat map analysis. Pathways were selected by corrected $P < 0.05$

each study varied. The higher abundances of *Haemophilus*, *Neisseria*, *Fusobacteria*, and *Porphyromonas* were detected in bronchial bacterial microbiota in adult asthma;²⁵ *Haemophilus influenzae* and *Tropheryma whippelii* were found abundant in induced sputum samples from asthmatic patients;²⁶ and *Moraxella catarrhalis*, *Haemophilus*, and *Streptococcus* were higher in the sputum of patients with severe asthma.²⁷ In this study, we found a higher abundance of uncultured *Corynebacteriales* in non-asthmatics than in asthmatics among young adults, whereas a higher abundance of *Moraxella* was seen in non-asthmatic subjects than in asthmatics among elderly individuals. The discrepancies among microbiome studies may be caused by the differences in sampling sites, analysis methods, age groups, severity, and clinical phenotypes of subjects with asthma. Asthma is heterogeneous in its

clinical presentation and severity;²⁸ thus, the microbiome for asthma should be analyzed by defined clinical groups. In the present study, the upper airway samples were analyzed in young adults and elderly with heterogeneous features of asthma, which were different from previous studies that showed various microbial associations.

We analyzed the association between genera and clinical features in both age groups (Figure 2). Different microbes were related to asthmatic features between young adults and elderly individuals. The high abundances of *Prevotella*, *Neisseria*, and *Fusobacterium* were related to asthmatic features in young adults. The higher abundances of *Neisseria* and *Fusobacterium* in asthmatics were detected in previous studies.^{25,29} Among elderly individuals, two genera (*Burkholderia* and *Psychrobacter*) within Proteobacteria and one genus

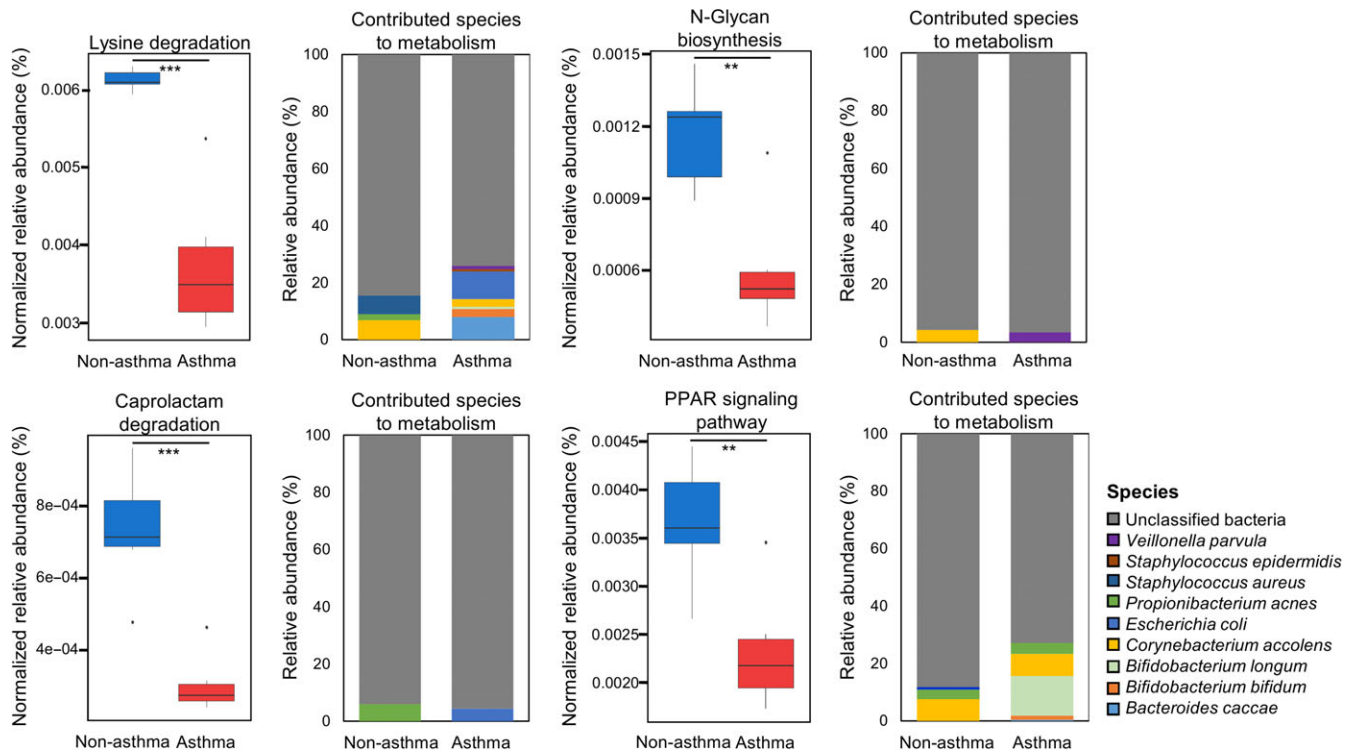


FIGURE 4 Comparison of normalized gene abundances and related species in metabolic pathway with higher abundance in non-asthmatics than asthmatics of young adults. KEGG categories of lysine degradation, N-glycan biosynthesis, caprolactam degradation, and PPAR signaling pathway were selected and compared between groups. (** $P < 0.01$; *** $P < 0.005$). Detailed gene family in each pathway is detailed in Table S5 [Colour figure can be viewed at [wileyonlinelibrary.com](#)]

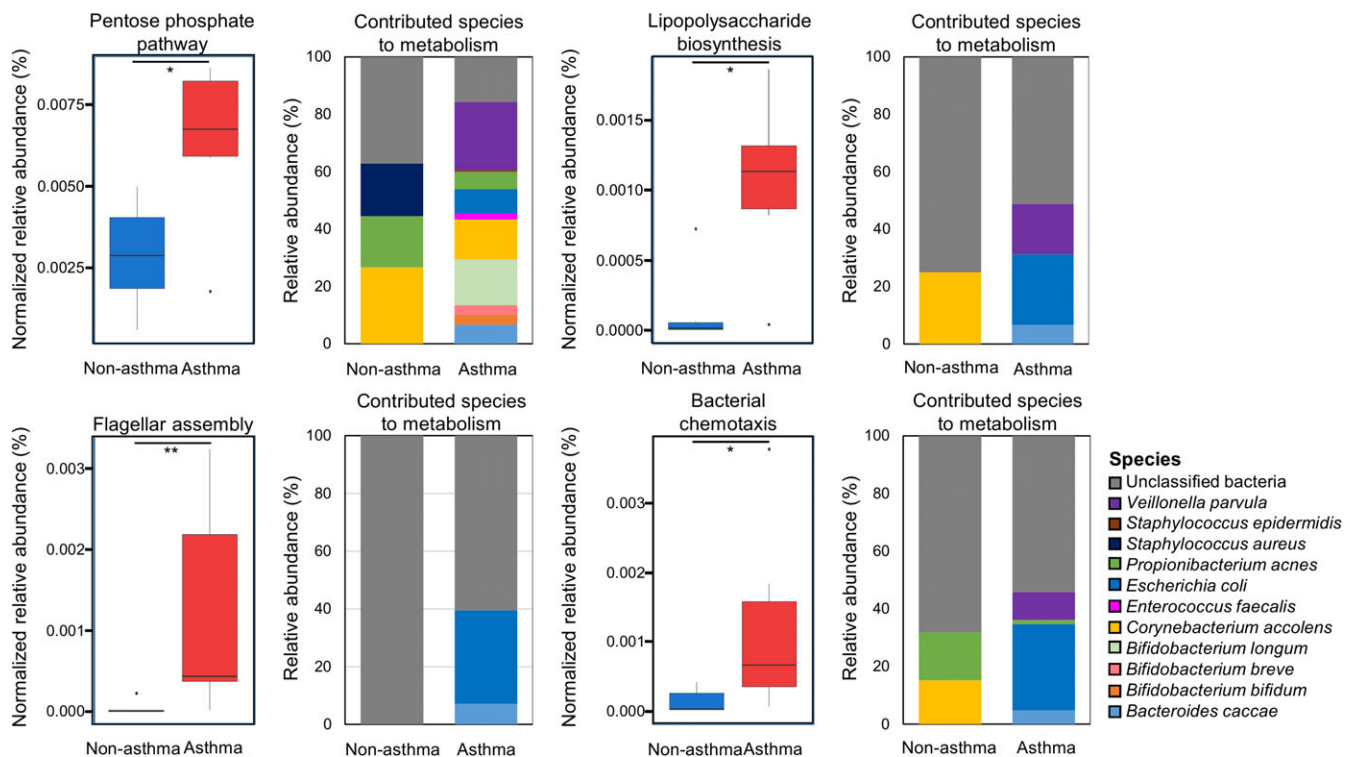


FIGURE 5 Comparison of normalized gene abundances and related species in metabolic pathway with higher abundance in asthmatics than non-asthmatics of young adults. KEGG categories of pentose phosphate pathway, lipopolysaccharide biosynthesis, flagellar assembly, and bacterial chemotaxis were selected and compared. (* $P < 0.05$; ** $P < 0.01$). Detailed gene family in each pathway is detailed in Table S6 [Colour figure can be viewed at [wileyonlinelibrary.com](#)]

(*Corynebacterium*) within Actinobacteria revealed contrary relationships with FEV₁. Low abundances of *Burkholderia* and *Psychrobacter*, whereas high abundance of *Corynebacterium* in the upper airways could be related to asthmatic features. Higher abundance of *Corynebacterium* was reported in the upper airways of patients with rhinosinusitis.³⁰ However, the association of specific bacteria with rhinosinusitis was not detected in the present study (data not shown). These results suggest that the upper airway microbiota could be associated with lung functions and asthma in young adults and elderly individuals.

Differences in the potential roles of airway microbiome in asthma were detected in young adults. The relative abundances of microbiome genes associated with lysine degradation, N-glycan biosynthesis, caprolactam degradation, and PPAR signaling pathway were lower in asthmatics than in non-asthmatics (Figure 4). Lysine residues can increase the activity of proinflammatory factors and affect collagen synthesis.¹⁹ Thus, lysine degradation can regulate airway inflammation and remodeling, which are key pathogenic features of asthma. This role of airway microbiome was also demonstrated in childhood asthma.¹⁷ Genes related to N-glycan biosynthesis were downregulated in the severe asthma group of transcriptome analysis.³¹ This pathway is responsible for the production of glycans, and the alteration of glycoproteins distribution can contribute to chronic diseases, including autoimmunity.³² Caprolactam is found in many indoor products, such as synthetic fibers, resins, synthetic leather, and plasticizers. Therefore, caprolactam degradation by airway microbiome could be related to the degradation of air pollutants found indoors.²⁰ PPAR- α and PPAR- γ modulate allergic inflammation and can reduce the levels of inflammatory cytokines.^{33,34} These pathway differences were caused by different contributed species in pathways. *C. accolens*, *P. acnes*, and *S. aureus* were mainly contributed to these pathways in non-asthmatic subjects.

On the other hand, the relative abundances of microbiome genes associated with the pentose phosphate pathway, lipopolysaccharide biosynthesis, flagellar assembly, and bacterial chemotaxis were higher in asthmatics than in non-asthmatics (Figure 5). Pentose phosphate pathway can reduce the capacity in erythrocyte and increase oxidant stress and asthma risk.³⁵ This pathway can mediate the production of bacterial hyaluronic acid (HA), and HA is related to the severity and inflammation in asthma.^{21,36} Bacterial lipopolysaccharide is a potential stimulator of nitric oxide (NO) production, and the increased level of NO was detected in asthmatic patients.^{37,38} Bacterial lipopolysaccharide could be related to asthma by suppressing Th2 responses through Toll-like receptor 4-dependent pathway, and the airway exposed level of lipopolysaccharide induced different forms of asthma phenotypes.³⁹ Bacterial flagellin stimulates strong allergic airway responses and promotes the development of allergic asthma by TLR5-dependent priming of allergic responses to indoor allergens.⁴⁰ Bacterial chemotaxis relates to the colonization of bacteria on epithelial surface and inflammation.²² Therefore, higher abundances of these pathways in asthmatics could suggest that the airway microbiome can promote airway

inflammation and development of asthma. These differences were also caused by different contributed bacteria affecting the pathways. *B. caccae*, *E. coli*, and *V. parvula* were main species contributing to these pathways. Our finding suggests that the functional genes of airway microbiome may play bi-directional roles in asthma pathogenesis, either promoting or preventing airway inflammation in young adults. However, this study showed no significantly different functional genes of airway microbiome between asthmatics and non-asthmatics in elderly individuals.

Recent literature indicates that asthma exhibits distinct clinical features depending on age.⁴¹ Compared to children or young adults, elderly asthmatic patients suffer greater morbidity and healthcare costs.^{41,42} According to Severe Asthma Research Program (SARP), asthma is more severe in older adults, and age seems to have a greater impact on the risk of asthma than disease duration.⁴³ Elderly patients are known to have greater small airway involvement and sputum neutrophilia.⁴⁴ Various factors, such as aging-related alteration in the lung and immunity, environmental exposures, microbial triggers, epigenetic changes, and comorbid conditions, might be involved in these pathophysiologic difference.⁴² In particular, the upper airway comorbid conditions are different between young adult asthmatics and elderly asthmatics. Asthma in young adults is closely associated with allergic rhinitis, whereas asthma in the elderly is associated with chronic rhinosinusitis or nasal polyp.^{11,42} Therefore, the upper airway microbiome associated with asthma of young adults could be different to elderly subjects.

There are several limitations to this study. Given the dynamic nature of microbiota in asthma, we were only able to include relatively small number of subjects in each group with a cross-sectional design at a single time point. Moreover, the influence of many confounding factors that induce lung dysbiosis, such as the effect of environmental factors, including indoor, outdoor pollution, occupation, habitation, and socioeconomic factors, was not included in the analysis. Biomarkers of airway inflammation other than peripheral blood eosinophil such as exhaled nitric oxide or sputum eosinophil were not fully assessed in this study. However, our analyzed sample size was comparable to previous studies, and the potential role of upper airway microbiome in this study provides valuable information for understanding the influence of upper airway microbiota on asthma. Although the ages between non-asthmatics and asthmatics differed in this study, the analyzed microbiota was not associated with age in each age group (Figure S2). In addition, the upper airway microbiome using a nasopharyngeal swab cannot represent the microbiome in the lower airway. However, the microbiota in the upper airway can harbor both pathogens and potentially pathogenic organisms, and may contribute to the dysbiosis of the lower airway through microaspiration and systemic routes.^{6,45} Furthermore, the nasopharyngeal swab is non-invasive for elderly individuals. Further studies with a larger sample size, metabolomics, and analyses of host-microbiome interaction and secondary confounding factors are needed to validate these findings. Such studies will elucidate the accurate roles of airway microbiome in the pathogenesis of asthma.

Alteration of airway microbiome can lead to perturbation and inflammation in the airway mucosa. The upper airway microbiome was different between young adults and elderly individuals, and their association with asthma was also different. We found the association of bacterial genera with clinical features in both age groups. The microbiome genes related to reducing airway inflammation and degrading indoor air pollutants were lower in asthmatics, whereas genes enhancing airway inflammation and mucosal bacterial colonization were higher in asthmatics among young adults. These results suggest that the alteration of upper airway microbiome can be associated with asthma through their functional roles and that elderly asthma have distinct pathogenicity from young adult asthma with respect to airway microbiome. Further studies on the role of detected pathways will be necessary to clarify the association of airway microbiome with asthma pathogenesis.

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CONFLICT OF INTEREST

There is no conflict of interest to declare for all authors.

AUTHOR CONTRIBUTIONS

S.J.H., Y.S.C., and B.S.K. conceived the study; J.J.L., S.H.K., and M.J.L. participated in investigation; J.J.L., M.J.L., and S.H.K. performed formal analysis; B.K.K., W.J.S., S.H.C., H.W.P., Y.S.C., and B.S.K. collected the resources; J.J.L., M.J.L., and B.S.K. curated the data; J.J.L., S.H.K., S.J.H., Y.S.C., and B.S.K. wrote the manuscript; and S.H.K., Y.S.C., and B.S.K. supervised the study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Supplementary appendices

METHODS

Study subjects and sample collection

Eighty subjects (30 asthmatic and 10 non-asthmatic young adults between the ages 18 and 45 years; 30 asthmatic and 10 non-asthmatic elderly individuals over the age of 65 years) were enrolled in this study. Patients with asthma were recruited with following inclusion criteria: (1) airway reversibility documented by an increase of forced expiratory volume in one second (FEV₁) of 12% and 200 mL after inhalation of 200 µg of salbutamol, or airway hyperresponsiveness documented by a methacholine provocation concentration causing a 20% fall in FEV₁ (PC20) of ≤16 mg/mL, in the past or at the time of enrollment; and (2) presence of persistent characteristic asthma symptoms and physician's diagnosis of asthma. The severity of asthma was assessed retrospectively from the level of treatment to control symptoms and exacerbations^{E1}. Mild asthma is asthma that can be controlled with Global Initiative for Asthma (GINA) step 1 or step 2 treatment; moderate asthma is one that can be controlled with GINA step 3 treatment. Severe asthma is asthma that requires step 4 or step 5 treatment to maintain symptom control. Subjects with any history of recent infection, use of antibiotics, acute exacerbation of asthma, and systemic steroid use during the previous 4 weeks before enrollment were excluded. Subjects with any uncontrolled serious diseases, such as malignancy or autoimmune diseases, any active pulmonary disease other than asthma, pregnancy and lactation were also excluded from the study. All subjects were recruited from Seoul National University Bundang Hospital and Seoul National University Hospital in Korea. Upper airway nasopharyngeal swab samples were collected at the subjects' nasopharyngeal wall using ESwabTM collection kits (Copan Diagnostic Inc, Murieta, USA) containing swab and transport medium as a manufacturer's instructions. The

nasopharyngeal swab samples were stored at -80°C immediately after sample collection until processed for DNA extraction. The clinical characteristics of patients with asthma were analyzed using their clinical information and laboratory findings, such as blood or sputum granulocyte profiles, spirometry profiles, exhaled nitric oxide levels, and imaging (Table S4). Written consent was obtained from all subjects, and the study was approved by the Institutional Review Board of Seoul National University Bundang Hospital (IRB number B-1512/328-304).

DNA extraction and pyrosequencing

Metagenomic DNA was extracted from nasopharyngeal swabs using a RNeasy PowerMicrobiome kit (Qiagen, Valencia, CA, USA). The extracted metagenomic DNA was amplified using the barcoded primers targeting V1-V3 regions of the 16S rRNA gene^{E2,3} and a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA). Negative controls (blank swab) were also included in both steps – extraction and PCR amplification – and evaluated by gel electrophoresis after each step. One sample of elderly subjects was excluded for further analysis due to failure of DNA extraction. Amplicons were purified using the QIAquick PCR purification kit (Qiagen) and quantified using the PicoGreen dsDNA Assay kit (Invitrogen, Carlsbad, CA, USA). Equimolar concentrations of each amplicon from different samples were pooled and sequenced on a Roche/454 GS FLX+ system according to the manufacturer's instructions (Roche, Branford, CT, USA).

Whole metagenome sequencing

A total of 24 samples from non-smokers were randomly selected from each group (6 non-asthmatic and 6 asthmatic young adults; 6 non-asthmatic and 6 asthmatic of elderly

individuals), and used for whole metagenome sequencing. The selected metagenomic DNA samples were fragmented using Covaris M220 sonicator (Covaris, Inc., Woburn, MA, USA), and the sequencing libraries were prepared using Truseq Nano DNA library prep kit, according to the manufacturer's protocol (Illumina, San Diego, CA, USA). The prepared libraries were evaluated for their sizes and qualities by Bioanalyser 2100 (Agilent Technologies, Santa Clara, CA, USA). The concentration of the library was measured using a PicoGreen dsDNA Assay kit (Invitrogen). Equimolar concentrations of each library (2 nM) were prepared by quantification using CFX 96 Real-Time System (Bio-Rad) with Illumina adapter sequence primers. Sequencing was performed using Illumina HiSeq 2500 platform (250 bp paired ends).

Analyses of microbiota and whole metagenome sequence

To analyze the composition of microbiota, sequences obtained from pyrosequencing were analyzed, as described previously^{E3,4}. Barcodes and primers in sequences were trimmed off, and low-quality reads ($Q < 25$ or read length < 300 bp) were removed. Chimeric sequences were detected and removed by UCHIME^{E5}. Sequences were clustered into operational taxonomic units (OTUs) at 97% similarity, and representative sequences from each cluster were taxonomically assigned using RDP classifier^{E6} with the EzTaxon-e database^{E7}. The diversity indices were calculated using Mothur program after normalizing the read number for each sample^{E8}. Principal coordinate analysis (PCoA) was performed to compare the microbiota among the groups, based on the Bray-Curtis distance using Calypso^{E9}.

For analyses of whole metagenome sequences, sequences obtained from the HiSeq 2500 were analyzed, as described previously^{E4,10}. In brief, raw sequences were trimmed using Nsoni clip tool to remove the adapters, and sequences with short length (< 150 bp in each pair) and low Q score ($Q < 15$). Contaminated human genes were removed using BBDMap

(<http://sourceforge.net/project/bbmap>) with a reference human genome. Functional profiling of metagenomic sequences was performed using HUMAnN2^{E11}, and the resulting profiles were obtained with respect to gene family abundance at the community level in reads per kilobase (RPK) to normalize for gene length. The resulting UniRef50 IDs were converted to KEGG Orthology (KO) based on the matching K numbers (<http://www.genome.jp/kegg/kegg2.html>) and compared among groups.

The sequencing reads obtained from the study are available in the Korean NIH database under accession numbers R000294 and R000314 (<http://coda.nih.go.kr>).

RESULTS

The associated microbiota members with asthma severity

The difference of airway microbiota according to severity was compared in each group (Figure S5). Although the diversity was not significantly different among each group, the composition of microbiota was different depending on severity. In the young adult group, the relative abundances of Proteobacteria were higher in moderate and severe groups of asthmatics than in non-asthmatics ($p < 0.05$). However, the relative abundance of uncultured *Corynebacteriales* was higher in non-asthmatics than those in all severity groups of asthmatics ($p < 0.05$). In the elderly group, the relative abundances of *Streptococcus* and *Flavobacterium* were different among groups according to the degree of asthma severity. The proportion of *Streptococcus* was higher in moderate asthmatics than in other groups ($p < 0.05$). The relative abundances of *Flavobacterium* were lower in mild asthmatics than in non-asthmatic controls and moderate asthmatics ($p < 0.05$).

Different functional genes of airway microbiome between asthmatics and non-

asthmatics

A total of 150,265,745 reads (250 bp paired end) were generated for whole metagenome sequences, and the average proportion of functional genes among groups was compared at the higher KEGG Orthology (KO) categories (Figure S6). All categories were significantly different between asthmatics and non-asthmatics among young adults according to Benjamini-Hochberg multiple testing correction (corrected $p < 0.05$). The mean value of normalized metagenomic genes in metabolism category was higher in asthmatics than in non-asthmatics, whereas the values in other categories were lower in asthmatics than in non-asthmatics. Contrastingly, there were no significant differences between asthmatics and non-asthmatics among elderly individuals (corrected $p > 0.05$).

The detected genefamilies within significantly different pathways between asthmatics and non-asthmatics among young adults were detailed in Table S5, S6, and S7.

DISCUSSION

Alterations of airway microbiome can occur depending on the severity of asthma. We compared the airway microbiota according to asthma severity in each group (Figure S4). Proteobacteria was increased in young adults with moderate and severe degrees of asthma, whereas *Streptococcus* was increased in elderly individuals with moderate degree of asthma. The higher abundance of *Streptococcus* in adults with severe asthma has been reported in previous studies^{E12,13}. Although the abundance of *Streptococcus* was not significantly different in the young adult group of our study, the association of *Streptococcus* with severity was detected in the elderly group. The difference of our data to previous studies could be attributed to the difference in the sampling sites and age of subjects.

The association of microbiota with clinical variables including smoking history, used

124 medicine, family history, and comorbidities were analyzed (data not shown). There were no
125 statistical differences of diversity and microbiota among comparing groups ($p > 0.05$). This
126 could be due to the relatively small number of subjects in each group according to clinical
127 variables.

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Supplementary Table S1. Summary of diversity indices after normalizing the read numbers of each sample.

Group	Sample	Analyzed reads	Normalized reads	Observed OTUs	Estimated OTUs (Chao1)	Shannon diversity index	Good's coverage
Young adults non- asthmatics	B3001	13,594	3,000	84	166.1	1.15	0.99
	B3002	14,289	3,000	94	164.8	1.13	0.98
	B3003	13,901	3,000	79	251.5	1.69	0.98
	B3004	9,422	3,000	118	206.5	1.99	0.98
	B3005	6,505	3,000	115	203.5	2.50	0.98
	B3006	8,869	3,000	124	242.2	2.64	0.98
	B3007	15,491	3,000	165	306.8	2.12	0.97
	B3008	12,411	3,000	118	215.5	2.15	0.98
	B3009	14,034	3,000	50	116.4	1.09	0.99
	B3010	3,735	3,000	102	184.5	1.92	0.98
Young adults asthmatics	B1001	20,609	3,000	77	106.1	2.05	0.99
	B1002	6,021	3,000	82	174.6	2.08	0.99
	B1003	15,575	3,000	80	163.2	1.66	0.98
	B1004	11,797	3,000	71	100.1	0.91	0.99
	B1005	14,051	3,000	72	159.9	1.50	0.99
	B1006	4,320	3,000	817	1,819.5	5.63	0.84
	B1007	16,157	3,000	87	299.1	1.36	0.98
	B1008	20,570	3,000	70	213.5	1.80	0.99
	B1009	18,187	3,000	106	307.0	1.54	0.98
	B1010	10,243	3,000	84	155.8	1.67	0.99
	B1011	10,735	3,000	122	270.6	2.20	0.98
	B1012	6,385	3,000	137	349.7	1.56	0.97
	B1013	19,932	3,000	30	60.6	0.22	0.99
	B1014	16,025	3,000	150	417.2	2.44	0.97
	B1015	11,466	3,000	171	310.5	3.00	0.97
	B1016	11,766	3,000	57	139.7	1.52	0.99
	B1017	17,851	3,000	105	211.4	2.16	0.98
	B1018	5,727	3,000	160	263.9	1.77	0.98
	B1019	12,795	3,000	67	116.6	1.42	0.99
	B1020	18,101	3,000	49	130.2	1.04	0.99
	B1021	14,632	3,000	114	306.9	1.74	0.98
	B1022	16,042	3,000	64	106.3	1.68	0.99
	B1023	14,424	3,000	113	211.3	2.15	0.98

	B1024	13,889	3,000	126	304.1	1.68	0.97
	B1025	12,881	3,000	74	114.1	1.83	0.99
	B1026	13,503	3,000	449	1,297.5	3.62	0.90
	B1027	12,866	3,000	268	447.1	3.77	0.96
	B1028	18,639	3,000	30	52.8	0.22	0.99
	B1029	14,034	3,000	87	202.0	1.79	0.98
	B1030	17,679	3,000	274	622.9	3.31	0.95
Elderly non- asthmatics	B4001	11,415	3,000	180	486.7	2.09	0.96
	B4002	23,802	3,000	115	249.0	1.74	0.98
	B4003	14,779	3,000	69	152.3	1.98	0.99
	B4004	22,429	3,000	94	164.5	1.69	0.98
	B4005	16,217	3,000	91	280.4	1.32	0.98
	B4006	21,565	3,000	102	330.8	1.68	0.98
	B4007	16,502	3,000	321	1,032.3	1.78	0.92
	B4008	9,847	3,000	147	546.7	0.83	0.96
	B4009	16,275	3,000	58	105.3	0.55	0.99
	B4010	8,891	3,000	111	282.1	2.28	0.98
Elderly asthmatics	B2001	10,084	3,000	58	157.2	0.62	0.99
	B2002	26,860	3,000	58	146.0	1.06	0.99
	B2003	15,799	3,000	62	492.5	0.50	0.99
	B2004	11,628	3,000	105	301.7	1.63	0.98
	B2005	29,786	3,000	42	84.9	0.47	0.99
	B2006	32,252	3,000	337	558.0	4.13	0.95
	B2007	9,855	3,000	878	1,968.3	5.60	0.81
	B2008	17,458	3,000	79	161.1	1.79	0.99
	B2009	11,428	3,000	214	335.3	2.23	0.97
	B2010	5,804	3,000	240	343.1	2.69	0.97
	B2011	15,906	3,000	72	195.5	1.74	0.99
	B2013	12,659	3,000	134	321.1	2.00	0.97
	B2014	15,364	3,000	255	422.2	3.64	0.96
	B2015	7,157	3,000	286	413.5	3.44	0.96
	B2016	5,861	3,000	408	655.8	3.63	0.94
	B2017	21,009	3,000	46	69.0	1.64	0.99
	B2018	10,884	3,000	298	545.7	2.92	0.95
	B2019	9,796	3,000	598	1,103.8	4.28	0.88
	B2020	21,117	3,000	68	115.6	0.95	0.99
	B2021	17,680	3,000	130	217.6	1.79	0.98
	B2022	12,554	3,000	253	433.8	2.94	0.96
	B2023	10,525	3,000	75	135.5	0.95	0.99

B2024	8,992	3,000	103	167.5	1.40	0.98
B2025	14,184	3,000	65	229.0	0.66	0.99
C2001	11,753	3,000	56	143.0	0.94	0.99
C2002	14,005	3,000	83	182.0	2.00	0.99
C2003	3,028	3,000	535	1,091.2	5.24	0.91
C2004	14,573	3,000	88	182.0	1.41	0.98
C2005	19,118	3,000	57	90.8	1.59	0.99

Supplementary Table S2. Detected KEGG Orthology (KO) categories of upper airway microbiome with higher abundance in non-asthmatics compared with asthmatics among young adults.

KEGG Orthology category			Normalized proportions of detected metagenomes				
1st category	2nd category	3rd category	Non-asthmatics	Asthmatics	Mean difference	p	Corrected p
Organismal Systems	Immune system	RIG-I-like receptor signaling pathway	0.0010 ± 0.0002	0.0005 ± 0.0002	0.0005	0.0022	0.0410
Environmental Information Processing	Signal transduction	AMPK signaling pathway	0.0042 ± 0.0007	0.0019 ± 0.0007	0.0022	0.0043	0.0410
Metabolism	Glycan biosynthesis and metabolism	Glycosaminoglycan degradation	0.0040 ± 0.0005	0.0025 ± 0.0006	0.0015	0.0043	0.0410
Metabolism	Amino acid metabolism	Tryptophan metabolism	0.0025 ± 0.0004	0.0013 ± 0.0005	0.0011	0.0043	0.0410
Metabolism	Lipid metabolism	Ether lipid metabolism	0.0007 ± 0.0002	0.0002 ± 0.0001	0.0004	0.0043	0.0410
Environmental Information Processing	Signal transduction	HIF-1 signaling pathway	0.0153 ± 0.0018	0.0081 ± 0.0027	0.0072	0.0087	0.0410
Organismal Systems	Endocrine system	Insulin signaling pathway	0.0152 ± 0.0026	0.0081 ± 0.0026	0.0072	0.0087	0.0410
Metabolism	Lipid metabolism	Arachidonic acid metabolism	0.0012 ± 0.0005	0.0005 ± 0.0001	0.0007	0.0087	0.0410
Organismal Systems	Immune system	Cytosolic DNA-sensing pathway	0.0010 ± 0.0003	0.0005 ± 0.0002	0.0006	0.0087	0.0410
Environmental Information Processing	Signal transduction	PI3K-Akt signaling pathway	0.0100 ± 0.0018	0.0053 ± 0.0023	0.0047	0.0152	0.0414
Human Diseases	Infectious diseases	Epstein-Barr virus infection	0.0079 ± 0.0014	0.0041 ± 0.0017	0.0039	0.0152	0.0414
Environmental	Signal	Phospholipase D	0.0037 ± 0.0008	0.0019 ± 0.0010	0.0018	0.0260	0.0469

Information Processing	transduction	signaling pathway					
Organismal Systems	Endocrine system	Estrogen signaling pathway	0.0039 ± 0.0007	0.0022 ± 0.0010	0.0017	0.0259	0.0469
Metabolism	Carbohydrate metabolism	Inositol phosphate metabolism	0.0027 ± 0.0005	0.0017 ± 0.0007	0.0010	0.0260	0.0469

Supplementary Table S3. Detected KO categories of upper airway microbiome with higher abundance in asthmatics compared with non-asthmatics among young adults.

KEGG Orthology category			Normalized proportions of detected metagenomes				
1st category	2nd category	3rd category	Non-asthmatics	Asthmatics	Mean difference	p	Corrected p
Metabolism	Glycan biosynthesis and metabolism	Peptidoglycan biosynthesis	0.0021 ± 0.0011	0.0076 ± 0.0024	0.0054	0.0043	0.0410
Metabolism	Lipid metabolism	Biosynthesis of unsaturated fatty acids	0.0006 ± 0.0003	0.0019 ± 0.0007	0.0013	0.0043	0.0410
Metabolism	Amino acid metabolism	Cysteine and methionine metabolism	0.0044 ± 0.0027	0.0133 ± 0.0044	0.0088	0.0087	0.0410
Metabolism	Metabolism of cofactors and vitamins	Vitamin B6 metabolism	0.0006 ± 0.0004	0.0017 ± 0.0006	0.0011	0.0087	0.0410
Metabolism	Metabolism of terpenoids and polyketides	Terpenoid backbone biosynthesis	0.0022 ± 0.0011	0.0036 ± 0.0006	0.0015	0.0152	0.0414
Metabolism	Overview	2-Oxocarboxylic acid metabolism	0.0038 ± 0.0022	0.0084 ± 0.0021	0.0046	0.0152	0.0414
Environmental Information Processing	Membrane transport	ABC transporters	0.0131 ± 0.0069	0.0426 ± 0.0156	0.0295	0.0152	0.0414
Metabolism	Metabolism of terpenoids and polyketides	Polyketide sugar unit biosynthesis	0.0001 ± 0.0002	0.0008 ± 0.0003	0.0006	0.0200	0.0469
Metabolism	Metabolism of cofactors and vitamins	Biotin metabolism	0.0010 ± 0.0008	0.0027 ± 0.0011	0.0016	0.0260	0.0469
Metabolism	Metabolism of cofactors and vitamins	Folate biosynthesis	0.0028 ± 0.0016	0.0045 ± 0.0016	0.0018	0.0260	0.0469

Metabolism	Metabolism of cofactors and vitamins	Nicotinate and nicotinamide metabolism	0.0018 ± 0.0013	0.0045 ± 0.0017	0.0027	0.0260	0.0469
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Supplementary Table S4. Summary of clinical characteristics of patients with asthma.

	Gender	Age	Smoking (pack year)	Asthma diagnosis			Asthma medication				ICS dose (daily, beclometasone equivalent, µg)
				MBPT (PC20)	Bronchodilator response (% ml)	FEV increase after anti- inflammatory treatment (% ml)	ICS	ICS/LABA	LTRA	xanthine	
Young adults											
1	F	35	never	0.08	14.0%, 320ml			Y	Y		500
2	M	43	current (8)	1.49				Y			320
3	F	26	never	1.97			Y				400
4	M	45	never	0.57				Y	Y	Y	800
5	M	37	past (30)	0.69				Y			400
6	F	44	never	0.26				Y	Y		1000
7	F	33	never	0.4				Y			800
8	F	22	never	0.51				Y	Y		1000
9	F	27	never	1.36				Y			500
10	F	29	never	13.1				Y			320
11	F	42	current (5)			14.4%, 380ml		Y	Y		1000
12	F	36	current (13)	1.61				Y			320
13	F	45	never	0.34					Y		0
14	F	20	never		36.0%, 400ml		Y		Y		400
15	M	28	never		41.5%, 950ml			Y	Y		320
16	F	36	never			15.9%, 440ml		Y	Y		320
17	M	36	current (2.5)	6.73	13.8%, 520ml			Y	Y		800
18	F	36	never	1.37				Y	Y		400
19	F	41	never	1.68	14.8%, 350ml		Y		Y		800

20	F	32	never	0.95				Y	Y		320
21	M	25	never		12.2%, 320ml		Y				400
22	F	39	current (1)			13.3%, 330ml		Y	Y		400
23	M	30	never	1.77			Y		Y		400
24	F	26	never	8.43			Y				800
25	M	36	past (10)	6.08				Y	Y		400
26	M	24	never	5.97			Y				800
27	F	43	never	0.01	31.9% (230ml)			Y			320
28	F	36	never	9.11			Y				500
29	M	33	never	0.61				Y			400
30	M	37	past (7.5)		13.6%. 350ml			Y	Y		320
<i>Elderly</i>											
31	M	69	never	9.2	18.2%, 380ml		Y				400
32	F	66	never	11.4				Y	Y		400
33	F	75	never			30.5%, 390ml		Y	Y		640
34	M	78	past (9)		14.7%, 260ml			Y	Y		1000
35	F	71	never	5.93				Y			320
36	F	77	never	7.08				Y			400
37	F	66	never	1.88			Y				200
38	M	78	never	4.88				Y			1000
39	F	67	never	0.33	16.4%, 230ml			Y			400
40	M	65	past (20)		24.5%, 390ml			Y	Y	Y	1000
41	F	74	never	8.37	17.6%, 250ml			Y	Y		400
42	F	72	never	1.97	17.8%, 230ml			Y			400
43	M	73	current (3.7)	12.5				Y	Y		400
44	M	69	past (60)		13.3%, 200ml			Y			1000
45	M	65	past (15)			20.7%, 440ml		Y	Y		1000
46	F	73	never	13.7				Y	Y		400
47	F	68	never			21.4%, 220ml		Y			1000

48	F	87	never			24.4%, 200ml		Y			1000
49	M	68	past (26)			29.2%, 590ml		Y			320
50	M	69	past (40)			40.6%, 820ml		Y			1000
51	F	72	never	3.49	12.5%, 200ml			Y			400
52	F	74	never	5.6	25.9%, 570ml			Y	Y		320
53	F	80	never	2.14				Y	Y		1000
54	F	66	never	3.77				Y			1000
55	M	77	never					Y			320
56	F	72	never			96.9%, 630ml		Y		Y	500
57	M	68	never			72.2%, 840ml		Y		Y	2000
58	M	76	never			22.3%, 500ml	Y				800
59	M	81	never			30.1%, 590ml		Y		Y	1000
60	F	77	never			133.3%, 800ml		Y		Y	1000

ICS: inhaled corticosteroid, ICS/LABA: inhaled corticosteroid + inhaled long-acting beta2 agonist, LTRA: leukotriene receptor antagonist

Supplementary Table S5. Detected KEGG Orthology genefamily within metabolic pathways with higher abundance in non-asthmatics than asthmatics of young adults (related to Figure 4)

KEGG Orthology		Normalized proportion of detected metagenomes	
K number	Definition	Non-asthmatics	Asthmatics
Lysine degradation			
K00022	3-hydroxyacyl-CoA dehydrogenase [EC:1.1.1.35]	0.000003	0.000002
K00128	aldehyde dehydrogenase (NAD+) [EC:1.2.1.3]	0.000337	0.000323
K00135	succinate-semialdehyde dehydrogenase (NADP+) [EC:1.2.1.16]	0.000206	0.000067
K00143	L-aminoadipate-semialdehyde dehydrogenase [EC:1.2.1.31]	0.000031	0.000000
K00149	aldehyde dehydrogenase family 9 member A1 [EC:1.2.1.47 1.2.1.3]	0.000015	0.000002
K00164	2-oxoglutarate dehydrogenase E1 component [EC:1.2.4.2]	0.000324	0.000149
K00252	glutaryl-CoA dehydrogenase [EC:1.3.99.7]	0.000134	0.000018
K00290	saccharopine dehydrogenase (NAD+, L-lysine forming) [EC:1.5.1.7]	0.000000	0.000010
K00471	gamma-butyrobetaine dioxygenase [EC:1.14.11.1]	0.000005	0.000000
K00473	procollagen-lysine,2-oxoglutarate 5-dioxygenase 1 [EC:1.14.11.4]	0.000016	0.000008
K00474	trimethyllysine dioxygenase [EC:1.14.11.8]	0.000009	0.000000
K00626	acetyl-CoA C-acetyltransferase [EC:2.3.1.9]	0.000185	0.000128
K00658	2-oxoglutarate dehydrogenase E2 component (dihydrolipoamide succinyltransferase) [EC:2.3.1.61]	0.000139	0.000240
K00824	D-alanine transaminase [EC:2.6.1.21]	0.000000	0.000030
K00825	kynurenine/2-aminoadipate aminotransferase [EC:2.6.1.7 2.6.1.39]	0.000040	0.000010
K01034	acetate CoA-transferase alpha subunit [EC:2.8.3.8]	0.000000	0.000033
K01582	lysine decarboxylase [EC:4.1.1.18]	0.000058	0.000062

K01692	enoyl-CoA hydratase [EC:4.2.1.17]	0.000062	0.000007
K01782	3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase [EC:1.1.1.35 4.2.1.17 5.1.2.3]	0.000000	0.000001
K01825	3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase / enoyl-CoA isomerase [EC:1.1.1.35 4.2.1.17 5.1.2.3 5.3.3.8]	0.000000	0.000012
K01843	lysine 2,3-aminomutase [EC:5.4.3.2]	0.000000	0.000261
K03894	aerobactin synthetase subunit alpha [EC:6.3.2.27]	0.000011	0.000003
K03895	aerobactin synthetase subunit beta [EC:6.3.2.27]	0.000106	0.000000
K03897	lysine N6-hydroxylase [EC:1.14.13.59]	0.000001	0.000027
K06101	histone-lysine N-methyltransferase ASH1L [EC:2.1.1.43]	0.000062	0.000024
K07511	enoyl-CoA hydratase [EC:4.2.1.17]	0.000007	0.000000
K07514	enoyl-CoA hydratase / 3-hydroxyacyl-CoA dehydrogenase / 3,2-trans-enoyl-CoA isomerase [EC:4.2.1.17 1.1.1.35 5.3.3.8]	0.000504	0.000227
K07515	enoyl-CoA hydratase / long-chain 3-hydroxyacyl-CoA dehydrogenase [EC:4.2.1.17 1.1.1.211]	0.000155	0.000043
K09186	histone-lysine N-methyltransferase MLL1 [EC:2.1.1.43]	0.000053	0.000029
K09187	histone-lysine N-methyltransferase MLL2 [EC:2.1.1.43]	0.000082	0.000077
K09188	histone-lysine N-methyltransferase MLL3 [EC:2.1.1.43]	0.000300	0.000179
K09189	histone-lysine N-methyltransferase MLL5 [EC:2.1.1.43]	0.000069	0.000030
K11419	histone-lysine N-methyltransferase SUV39H [EC:2.1.1.43]	0.000028	0.000026
K11420	euchromatic histone-lysine N-methyltransferase [EC:2.1.1.43]	0.000014	0.000005
K11421	histone-lysine N-methyltransferase SETDB [EC:2.1.1.43]	0.000016	0.000024
K11422	histone-lysine N-methyltransferase SETD1 [EC:2.1.1.43]	0.000194	0.000040
K11423	histone-lysine N-methyltransferase SETD2 [EC:2.1.1.43]	0.000077	0.000031
K11424	histone-lysine N-methyltransferase NSD1/2 [EC:2.1.1.43]	0.000021	0.000009
K11425	histone-lysine N-methyltransferase NSD3 [EC:2.1.1.43]	0.000085	0.000111
K11427	histone-lysine N-methyltransferase, H3 lysine-79 specific [EC:2.1.1.43]	0.000020	0.000010

K11428	histone-lysine N-methyltransferase SETD8 [EC:2.1.1.43]	0.000099	0.000024
K11429	histone-lysine N-methyltransferase SUV420H [EC:2.1.1.43]	0.000008	0.000016
K11433	histone-lysine N-methyltransferase SETMAR [EC:2.1.1.43]	0.002437	0.001323
K11703	collagen beta-1,O-galactosyltransferase [EC:2.4.1.50]	0.000009	0.000002
K13645	procollagen-lysine,2-oxoglutarate 5-dioxygenase 2 [EC:1.14.11.4]	0.000027	0.000015
K14085	aldehyde dehydrogenase family 7 member A1 [EC:1.2.1.31 1.2.1.8 1.2.1.3]	0.000010	0.000003
K14157	alpha-aminoadipic semialdehyde synthase [EC:1.5.1.8 1.5.1.9]	0.000025	0.000004
K14268	5-aminovalerate aminotransferase [EC:2.6.1.48]	0.000000	0.000056
K14959	histone-lysine N-methyltransferase MLL4 [EC:2.1.1.43]	0.000014	0.000008
K15588	histone-lysine N-methyltransferase NSD1 [EC:2.1.1.43]	0.000024	0.000002
K18012	L-erythro-3,5-diaminohexanoate dehydrogenase [EC:1.4.1.11]	0.000020	0.000004
K18013	3-keto-5-aminohexanoate cleavage enzyme [EC:2.3.1.247]	0.000002	0.000000
K18201	hydroxylysine kinase [EC:2.7.1.81]	0.000043	0.000010
K18202	5-phosphonooxy-L-lysine phospho-lyase [EC:4.2.3.134]	0.000005	0.000002
K18494	histone-lysine N-methyltransferase SETDB2 [EC:2.1.1.43]	0.000018	0.000009
K18826	calmodulin-lysine N-methyltransferase [EC:2.1.1.60]	0.000000	0.000004

N-Glycan biosynthesis

K00717	glycoprotein 6-alpha-L-fucosyltransferase [EC:2.4.1.68]	0.000146	0.000022
K00721	dolichol-phosphate mannosyltransferase [EC:2.4.1.83]	0.000452	0.000028
K00726	alpha-1,3-mannosyl-glycoprotein beta-1,2-N-acetylglucosaminyltransferase [EC:2.4.1.101]	0.000135	0.000000
K00736	alpha-1,6-mannosyl-glycoprotein beta-1,2-N-acetylglucosaminyltransferase [EC:2.4.1.143]	0.000223	0.000031
K00737	beta-1,4-mannosyl-glycoprotein beta-1,4-N-acetylglucosaminyltransferase [EC:2.4.1.144]	0.000000	0.000009
K00738	alpha-1,3-mannosylglycoprotein beta-1,4-N-acetylglucosaminyltransferase A/B [EC:2.4.1.145]	0.000048	0.000004
K00744	alpha-1,3(6)-mannosylglycoprotein beta-1,6-N-acetyl-glucosaminyltransferase [EC:2.4.1.155]	0.000118	0.000014

K00778	beta-galactoside alpha-2,6-sialyltransferase (sialyltransferase 1) [EC:2.4.99.1]	0.000000	0.000004
K00779	beta-galactoside alpha2,6-sialyltransferase (sialyltransferase 2) [EC:2.4.99.1]	0.000035	0.000008
K01001	UDP-N-acetylglucosamine--dolichyl-phosphate N-acetylglucosaminephosphotransferase [EC:2.7.8.15]	0.000525	0.000052
K01228	mannosyl-oligosaccharide glucosidase [EC:3.2.1.106]	0.000270	0.000000
K01230	mannosyl-oligosaccharide alpha-1,2-mannosidase [EC:3.2.1.113]	0.000466	0.000040
K01231	alpha-mannosidase II [EC:3.2.1.114]	0.000079	0.000019
K03842	beta-1,4-mannosyltransferase [EC:2.4.1.142]	0.000663	0.000044
K03843	alpha-1,3/alpha-1,6-mannosyltransferase [EC:2.4.1.132 2.4.1.-]	0.000045	0.000004
K03844	alpha-1,2-mannosyltransferase [EC:2.4.1.-]	0.000283	0.000012
K03845	alpha-1,3-mannosyltransferase [EC:2.4.1.258]	0.000485	0.000017
K03846	alpha-1,2-mannosyltransferase [EC:2.4.1.259 2.4.1.261]	0.000226	0.000027
K03848	alpha-1,3-glucosyltransferase [EC:2.4.1.-]	0.000034	0.000000
K03849	alpha-1,3-glucosyltransferase [EC:2.4.1.-]	0.000148	0.000008
K03850	alpha-1,2-glucosyltransferase [EC:2.4.1.-]	0.000000	0.000017
K05546	alpha 1,3-glucosidase [EC:3.2.1.84]	0.000029	0.000000
K07151	dolichyl-diphosphooligosaccharide--protein glycosyltransferase [EC:2.4.1.119]	0.001144	0.000116
K07432	beta-1,4-N-acetylglucosaminyltransferase [EC:2.4.1.141]	0.000137	0.000047
K07441	beta-1,4-N-acetylglucosaminyltransferase [EC:2.4.1.141]	0.000047	0.000000
K07966	beta-1,4-galactosyltransferase 1 [EC:2.4.1.22 2.4.1.90 2.4.1.38 2.4.1.-]	0.000060	0.000000
K07967	beta-1,4-galactosyltransferase 2 [EC:2.4.1.22 2.4.1.90 2.4.1.38 2.4.1.-]	0.000033	0.000001
K07968	beta-1,4-galactosyltransferase 3 [EC:2.4.1.90 2.4.1.38 2.4.1.-]	0.000250	0.000015
K09658	dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit	0.000013	0.000000
K09661	mannosyl alpha-1,6-glycoprotein beta-1,6-N-acetyl-glucosaminyltransferase, isozyme B [EC:2.4.1.-]	0.000111	0.000014
K12666	oligosaccharyltransferase complex subunit alpha (ribophorin I)	0.000190	0.000006

K12667	oligosaccharyltransferase complex subunit delta (ribophorin II)	0.000097	0.000029
K12670	oligosaccharyltransferase complex subunit beta	0.000404	0.000008
K13748	alpha-1,3-mannosylglycoprotein beta-1,4-N-acetylglucosaminyltransferase C [EC:2.4.1.201 2.4.1.145]	0.000106	0.000001
Caprolactam degradation			
K00002	alcohol dehydrogenase (NADP+) [EC:1.1.1.2]	0.000000	0.000009
K00022	3-hydroxyacyl-CoA dehydrogenase [EC:1.1.1.35]	0.000003	0.000002
K01053	gluconolactonase [EC:3.1.1.17]	0.000001	0.000000
K01453	6-aminohexanoate-oligomer exohydrolase [EC:3.5.1.46]	0.000000	0.000001
K01692	enoyl-CoA hydratase [EC:4.2.1.17]	0.000062	0.000007
K01782	3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase [EC:1.1.1.35 4.2.1.17 5.1.2.3]	0.000000	0.000001
K01825	3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase / enoyl-CoA isomerase [EC:1.1.1.35 4.2.1.17 5.1.2.3 5.3.3.8]	0.000000	0.000012
K06446	acyl-CoA dehydrogenase [EC:1.3.99.-]	0.000000	0.000000
K07511	enoyl-CoA hydratase [EC:4.2.1.17]	0.000007	0.000000
K07514	enoyl-CoA hydratase / 3-hydroxyacyl-CoA dehydrogenase / 3,2-trans-enoyl-CoA isomerase [EC:4.2.1.17 1.1.1.35 5.3.3.8]	0.000504	0.000227
K07515	enoyl-CoA hydratase / long-chain 3-hydroxyacyl-CoA dehydrogenase [EC:4.2.1.17 1.1.1.211]	0.000155	0.000043
PPAR signaling pathway			
K00232	acyl-CoA oxidase [EC:1.3.3.6]	0.000018	0.000002
K00249	acyl-CoA dehydrogenase [EC:1.3.99.3]	0.000073	0.000046
K00255	long-chain-acyl-CoA dehydrogenase [EC:1.3.99.13]	0.000011	0.000007
K00489	cytochrome P450, family 7, subfamily A (cholesterol 7alpha-monooxygenase) [EC:1.14.13.17]	0.000005	0.000000
K00507	stearoyl-CoA desaturase (delta-9 desaturase) [EC:1.14.19.1]	0.000003	0.000000

K00864	glycerol kinase [EC:2.7.1.30]	0.000210	0.000076
K01388	matrix metalloproteinase-1 (interstitial collagenase) [EC:3.4.24.7]	0.000045	0.000056
K01596	phosphoenolpyruvate carboxykinase (GTP) [EC:4.1.1.32]	0.000052	0.000046
K01897	long-chain acyl-CoA synthetase [EC:6.2.1.3]	0.000344	0.000588
K04504	peroxisome proliferator-activated receptor delta	0.000051	0.000027
K06086	sorbin and SH3 domain containing 1	0.000010	0.000006
K06259	CD36 antigen	0.000005	0.000001
K06272	integrin-linked kinase [EC:2.7.11.1]	0.000051	0.000045
K06276	3-phosphoinositide dependent protein kinase-1 [EC:2.7.11.1]	0.000023	0.000001
K07294	peroxisome proliferator-activated receptor alpha	0.000037	0.000023
K07296	adiponectin	0.000010	0.000000
K07425	cytochrome P450, family 4, subfamily A [EC:1.14.15.3]	0.000012	0.000012
K07513	acetyl-CoA acyltransferase 1 [EC:2.3.1.16]	0.000000	0.000002
K07514	enoyl-CoA hydratase / 3-hydroxyacyl-CoA dehydrogenase / 3,2-trans-enoyl-CoA isomerase [EC:4.2.1.17 1.1.1.35 5.3.3.8]	0.000504	0.000227
K08524	retinoid X receptor alpha	0.000072	0.000043
K08525	retinoid X receptor beta	0.000023	0.000010
K08530	peroxisome proliferator-activated receptor gamma	0.000006	0.000004
K08745	solute carrier family 27 (fatty acid transporter), member 1/4 [EC:6.2.1.-]	0.000008	0.000005
K08746	solute carrier family 27 (fatty acid transporter), member 2 [EC:6.2.1.- 6.2.1.3]	0.000046	0.000005
K08749	solute carrier family 27 (fatty acid transporter), member 6	0.000072	0.000006
K08751	fatty acid-binding protein 2, intestinal	0.000019	0.000010
K08752	fatty acid-binding protein 3, muscle and heart	0.000001	0.000024
K08753	fatty acid-binding protein 4, adipocyte	0.000001	0.000024
K08754	fatty acid-binding protein 5, epidermal	0.000048	0.000003
K08756	fatty acid-binding protein 7, brain	0.000001	0.000024

K08757	apolipoprotein A-I	0.000007	0.000000
K08758	apolipoprotein A-II	0.000068	0.000009
K08762	diazepam-binding inhibitor (GABA receptor modulator, acyl-CoA-binding protein)	0.000012	0.000017
K08764	sterol carrier protein 2 [EC:2.3.1.176]	0.000044	0.000045
K08765	carnitine O-palmitoyltransferase 1 [EC:2.3.1.21]	0.000032	0.000027
K08766	carnitine O-palmitoyltransferase 2 [EC:2.3.1.21]	0.000011	0.000000
K08768	perilipin	0.000005	0.000009
K08769	solute carrier family 25 (mitochondrial uncoupling protein), member 7	0.000052	0.000011
K08770	ubiquitin C	0.001256	0.000722
K08771	aquaporin-7	0.000317	0.000113
K10214	3alpha,7alpha,12alpha-trihydroxy-5beta-cholestanoyl-CoA 24-hydroxylase [EC:1.17.99.3]	0.000018	0.000000
K10226	fatty acid desaturase 2 (delta-6 desaturase) [EC:1.14.19.-]	0.000005	0.000018
K15013	long-chain-fatty-acid--CoA ligase ACSBG [EC:6.2.1.3]	0.000020	0.000009
K17284	perilipin-2	0.000029	0.000000
K19524	carnitine O-palmitoyltransferase 1, brain isoform [EC:2.3.1.21]	0.000019	0.000019

Supplementary Table S6. Detected KEGG Orthology genefamily within metabolic pathways with higher abundance in asthmatics than non-asthmatics of young adults (related to Figure 5).

KEGG Orthology		Normalized proportion of detected metagenomes	
K number	Definition	Non-asthmatics	Asthmatics
Pentose phosphate pathway			
K00033	6-phosphogluconate dehydrogenase [EC:1.1.1.44]	0.000051	0.000281
K00036	glucose-6-phosphate 1-dehydrogenase [EC:1.1.1.49]	0.000179	0.000184
K00090	gluconate 2-dehydrogenase [EC:1.1.1.215]	0.000000	0.000024
K00117	quinoprotein glucose dehydrogenase [EC:1.1.5.2]	0.000000	0.000026
K00131	glyceraldehyde-3-phosphate dehydrogenase (NADP) [EC:1.2.1.9]	0.000000	0.000020
K00615	transketolase [EC:2.2.1.1]	0.000254	0.000228
K00616	transaldolase [EC:2.2.1.2]	0.000117	0.000195
K00850	6-phosphofructokinase [EC:2.7.1.11]	0.000272	0.000139
K00851	gluconokinase [EC:2.7.1.12]	0.000156	0.000035
K00852	ribokinase [EC:2.7.1.15]	0.000120	0.000461
K00874	2-dehydro-3-deoxygluconokinase [EC:2.7.1.45]	0.000000	0.000197
K00895	pyrophosphate--fructose-6-phosphate 1-phosphotransferase [EC:2.7.1.90]	0.000036	0.000029
K00948	ribose-phosphate pyrophosphokinase [EC:2.7.6.1]	0.000072	0.000852
K01053	gluconolactonase [EC:3.1.1.17]	0.000001	0.000000
K01057	6-phosphogluconolactonase [EC:3.1.1.31]	0.000067	0.001590
K01619	deoxyribose-phosphate aldolase [EC:4.1.2.4]	0.000508	0.000213
K01621	phosphoketolase [EC:4.1.2.9]	0.000000	0.000000

K01623	fructose-bisphosphate aldolase, class I [EC:4.1.2.13]	0.000046	0.000064
K01624	fructose-bisphosphate aldolase, class II [EC:4.1.2.13]	0.000158	0.000123
K01625	2-dehydro-3-deoxyphosphogluconate aldolase / 4-hydroxy-2-oxoglutarate aldolase [EC:4.1.2.14 4.1.3.16]	0.000094	0.000116
K01690	phosphogluconate dehydratase [EC:4.2.1.12]	0.000000	0.000020
K01783	ribulose-phosphate 3-epimerase [EC:5.1.3.1]	0.000030	0.000440
K01807	ribose 5-phosphate isomerase A [EC:5.3.1.6]	0.000077	0.000206
K01808	ribose 5-phosphate isomerase B [EC:5.3.1.6]	0.000158	0.000080
K01810	glucose-6-phosphate isomerase [EC:5.3.1.9]	0.000185	0.000282
K01835	phosphoglucomutase [EC:5.4.2.2]	0.000059	0.000280
K01839	phosphopentomutase [EC:5.4.2.7]	0.000038	0.000112
K02446	fructose-1,6-bisphosphatase II [EC:3.1.3.11]	0.000090	0.000048
K03841	fructose-1,6-bisphosphatase I [EC:3.1.3.11]	0.000001	0.000002
K04041	fructose-1,6-bisphosphatase III [EC:3.1.3.11]	0.000015	0.000016
K05774	ribose 1,5-bisphosphokinase [EC:2.7.4.23]	0.000000	0.000001
K07404	6-phosphogluconolactonase [EC:3.1.1.31]	0.000001	0.000003
K08093	3-hexulose-6-phosphate synthase [EC:4.1.2.43]	0.000046	0.000000
K08094	6-phospho-3-hexuloisomerase [EC:5.3.1.27]	0.000000	0.000021
K11645	2-amino-3,7-dideoxy-D-threo-hept-6-ulosonic acid synthase [EC:4.1.2.-]	0.000000	0.000003
K13810	transaldolase / glucose-6-phosphate isomerase [EC:2.2.1.2 5.3.1.9]	0.000000	0.000000
K13937	hexose-6-phosphate dehydrogenase [EC:1.1.1.47 3.1.1.31]	0.000015	0.000000
K15778	phosphomannomutase / phosphoglucomutase [EC:5.4.2.8 5.4.2.2]	0.000002	0.000018
K15779	phosphoglucomutase / phosphopentomutase [EC:5.4.2.2 5.4.2.7]	0.000031	0.000021
K16370	6-phosphofructokinase 2 [EC:2.7.1.11]	0.000000	0.000031

Lipopolysaccharide biosynthesis

K00677	UDP-N-acetylglucosamine acyltransferase [EC:2.3.1.129]	0.000000	0.000032
K00748	lipid-A-disaccharide synthase [EC:2.4.1.182]	0.000000	0.000041
K00912	tetraacyldisaccharide 4'-kinase [EC:2.7.1.130]	0.000000	0.000227
K00979	3-deoxy-manno-octulosonate cytidyltransferase (CMP-KDO synthetase) [EC:2.7.7.38]	0.000000	0.000062
K01627	2-dehydro-3-deoxyphosphooctonate aldolase (KDO 8-P synthase) [EC:2.5.1.55]	0.000000	0.000045
K02517	lipid A biosynthesis lauroyl acyltransferase [EC:2.3.1.-]	0.000121	0.000099
K02527	3-deoxy-D-manno-octulosonic-acid transferase [EC:2.-.-.]	0.000000	0.000021
K02535	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase [EC:3.5.1.-]	0.000000	0.000036
K02536	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase [EC:2.3.1.-]	0.000008	0.000138
K02560	lipid A biosynthesis (KDO)2-(lauroyl)-lipid iva acyltransferase [EC:2.3.1.-]	0.000000	0.000028
K02840	UDP-D-galactose:(glucosyl)LPS alpha-1,6-D-galactosyltransferase [EC:2.4.1.-]	0.000000	0.000013
K02841	heptosyltransferase I [EC:2.4.-.]	0.000000	0.000036
K02843	heptosyltransferase II [EC:2.4.-.]	0.000000	0.000009
K02844	UDP-glucose:(heptosyl)LPS alpha-1,3-glucosyltransferase [EC:2.4.1.-]	0.000000	0.000026
K02847	O-antigen ligase [EC:6.-.-.]	0.000000	0.000023
K02848	heptose (I) phosphotransferase [EC:2.7.1.-]	0.000000	0.000002
K02849	heptosyltransferase III [EC:2.4.-.]	0.000000	0.000043
K03269	UDP-2,3-diacylglucosamine hydrolase [EC:3.6.1.-]	0.000000	0.000005
K03270	3-deoxy-D-manno-octulosonate 8-phosphate phosphatase (KDO 8-P phosphatase) [EC:3.1.3.45]	0.000004	0.000040
K03271	phosphoheptose isomerase [EC:5.-.-.]	0.000000	0.000005
K03272	D-beta-D-heptose 7-phosphate kinase / D-beta-D-heptose 1-phosphate adenosyltransferase [EC:2.7.1.- 2.7.7.-]	0.000000	0.000011
K03273	D-glycero-D-manno-heptose 1,7-bisphosphate phosphatase [EC:3.1.3.-]	0.000000	0.000004
K03274	ADP-L-glycero-D-manno-heptose 6-epimerase [EC:5.1.3.20]	0.000000	0.000021
K03275	UDP-glucose:(glucosyl)LPS alpha-1,3-glucosyltransferase [EC:2.4.1.-]	0.000000	0.000000

K03276	UDP-glucose/galactose:(glucosyl)LPS alpha-1,2-glucosyl/galactosyltransferase [EC:2.4.1.-]	0.000000	0.000025
K03277	heptosyltransferase IV [EC:2.4.-.-]	0.000000	0.000005
K03279	UDP-glucose:(galactosyl)LPS alpha-1,2-glucosyltransferase [EC:2.4.1.58]	0.000000	0.000004
K03280	UDP-N-acetylglucosamine:(glucosyl)LPS alpha-1,2-N-acetylglucosaminyltransferase [EC:2.4.1.56]	0.000000	0.000002
K06041	arabinose-5-phosphate isomerase [EC:5.3.1.13]	0.000000	0.000006
K12975	phosphoethanolamine transferase	0.000000	0.000021
K16363	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase / 3-hydroxyacyl-[acyl-carrier-protein] dehydratase [EC:3.5.1.108 4.2.1.59]	0.000003	0.000022

Flagellar assembly

K02386	flagella basal body P-ring formation protein FlgA	0.000000	0.000062
K02387	flagellar basal-body rod protein FlgB	0.000000	0.000093
K02388	flagellar basal-body rod protein FlgC	0.000000	0.000014
K02389	flagellar basal-body rod modification protein FlgD	0.000000	0.000022
K02390	flagellar hook protein FlgE	0.000000	0.000025
K02391	flagellar basal-body rod protein FlgF	0.000000	0.000026
K02392	flagellar basal-body rod protein FlgG	0.000000	0.000128
K02393	flagellar L-ring protein precursor FlgH	0.000000	0.000007
K02396	flagellar hook-associated protein 1 FlgK	0.000000	0.000029
K02397	flagellar hook-associated protein 3 FlgL	0.000000	0.000288
K02398	negative regulator of flagellin synthesis FlgM	0.000000	0.000002
K02400	flagellar biosynthesis protein FlhA	0.000000	0.000064
K02401	flagellar biosynthetic protein FlhB	0.000000	0.000028
K02402	flagellar transcriptional activator FlhC	0.000000	0.000039
K02403	flagellar transcriptional activator FlhD	0.000000	0.000001

K02406	flagellin	0.000001	0.000023
K02407	flagellar hook-associated protein 2	0.000000	0.000034
K02408	flagellar hook-basal body complex protein FliE	0.000000	0.000002
K02409	flagellar M-ring protein FliF	0.000000	0.000023
K02411	flagellar assembly protein FliH	0.000002	0.000029
K02412	flagellum-specific ATP synthase [EC:3.6.3.14]	0.000000	0.000037
K02414	flagellar hook-length control protein FliK	0.000000	0.000046
K02416	flagellar motor switch protein FliM	0.000000	0.000026
K02417	flagellar motor switch protein FliN/FliY	0.000000	0.000008
K02419	flagellar biosynthetic protein FliP	0.000000	0.000009
K02420	flagellar biosynthetic protein FliQ	0.000000	0.000002
K02421	flagellar biosynthetic protein FliR	0.000038	0.000022
K02422	flagellar protein FliS	0.000000	0.000037
K02556	chemotaxis protein MotA	0.000000	0.000013
K02557	chemotaxis protein MotB	0.000000	0.000068

Bacterial chemotaxis

K00575	chemotaxis protein methyltransferase CheR [EC:2.1.1.80]	0.000008	0.000045
K02416	flagellar motor switch protein FliM	0.000000	0.000026
K02417	flagellar motor switch protein FliN/FliY	0.000000	0.000008
K02556	chemotaxis protein MotA	0.000000	0.000013
K02557	chemotaxis protein MotB	0.000000	0.000068
K03406	methyl-accepting chemotaxis protein	0.000000	0.000005
K03407	two-component system, chemotaxis family, sensor kinase CheA [EC:2.7.13.3]	0.000008	0.000055
K03408	purine-binding chemotaxis protein CheW	0.000000	0.000005

K03412	two-component system, chemotaxis family, response regulator CheB [EC:3.1.1.61]	0.000000	0.000007
K03413	two-component system, chemotaxis family, response regulator CheY	0.000000	0.000115
K03776	aerotaxis receptor	0.000055	0.000046
K05874	methyl-accepting chemotaxis protein I, serine sensor receptor	0.000000	0.000089
K05875	methyl-accepting chemotaxis protein II, aspartate sensor receptor	0.000000	0.000067
K05876	methyl-accepting chemotaxis protein III, ribose and galactose sensor receptor	0.000000	0.000002
K05877	methyl-accepting chemotaxis protein IV, peptide sensor receptor	0.000000	0.000066
K10108	maltose/maltodextrin transport system substrate-binding protein	0.000000	0.000366
K10439	ribose transport system substrate-binding protein	0.000065	0.000180
K10540	methyl-galactoside transport system substrate-binding protein	0.000000	0.000025
K12368	dipeptide transport system substrate-binding protein	0.000000	0.000027

Supplementary Table S7. Detected KEGG Orthology genefamily within arachidonic acid metabolism between non-asthmatics and asthmatics of young adults.

KEGG Orthology		Normalized proportion of detected metagenomes	
K number	Definition	Non-asthmatics	Asthmatics
K00079	carbonyl reductase (NADPH) [EC:1.1.1.184]	0.0000505	0.0000042
K00081	prostaglandin-E2 9-reductase [EC:1.1.1.189]	0.0001022	0.0000011
K00084	15-hydroxyprostaglandin dehydrogenase (NADP) [EC:1.1.1.197]	0.0000160	0.0000022
K00432	glutathione peroxidase [EC:1.11.1.9]	0.0000260	0.0000656
K00458	arachidonate 12-lipoxygenase [EC:1.13.11.31]	0.0000000	0.0000137
K00460	arachidonate 15-lipoxygenase [EC:1.13.11.33]	0.0000000	0.0000079
K00461	arachidonate 5-lipoxygenase [EC:1.13.11.34]	0.0001188	0.0000027
K00807	leukotriene-C4 synthase [EC:4.4.1.20]	0.0000069	0.0000000
K01047	phospholipase A2 [EC:3.1.1.4]	0.0000000	0.0000248
K01058	phospholipase A1 [EC:3.1.1.32]	0.0000037	0.0000286
K01254	leukotriene-A4 hydrolase [EC:3.3.2.6]	0.0000000	0.0000098
K01831	cytochrome P450, family 8, subfamily A (Prostacyclin synthase) [EC:5.3.99.4]	0.0000053	0.0000000
K01832	cytochrome P450, family 5, subfamily A (thromboxane-A synthase) [EC:5.3.99.5]	0.0000071	0.0000049
K05309	prostaglandin-E synthase [EC:5.3.99.3]	0.0000139	0.0000034
K07412	cytochrome P450, family 2, subfamily B [EC:1.14.14.1]	0.0000540	0.0000248
K07413	cytochrome P450, family 2, subfamily C [EC:1.14.14.1]	0.0000029	0.0000200
K07418	cytochrome P450, family 2, subfamily J [EC:1.14.14.1]	0.0001842	0.0000045
K07422	cytochrome P450, family 2, subfamily U	0.0003039	0.0000000
K07425	cytochrome P450, family 4, subfamily A [EC:1.14.15.3]	0.0000247	0.0000121

K08021	arachidonate 12-lipoxygenase (R-type) [EC:1.13.11.-]	0.0000077	0.0000198
K08022	arachidonate 15-lipoxygenase (second type) / 8-lipoxygenase (S-type) [EC:1.13.11.33 1.13.11.-]	0.0000120	0.0000011
K08726	soluble epoxide hydrolase [EC:3.3.2.10]	0.0000120	0.0000000
K14621	phospholipase B1, membrane-associated [EC:3.1.1.4 3.1.1.5]	0.0000133	0.0000284
K15730	cytosolic prostaglandin-E synthase [EC:5.3.99.3]	0.0000465	0.0000490
K16342	cytosolic phospholipase A2 [EC:3.1.1.4]	0.0000327	0.0000390
K16343	calcium-independent phospholipase A2 [EC:3.1.1.4]	0.0000085	0.0000000
K17687	alkane 1-monooxygenase [EC:1.14.15.3]	0.0000106	0.0000121
K17709	cytochrome P450 family 2 subfamily B polypeptide 6 [EC:1.14.13.-]	0.0000272	0.0000045
K17719	cytochrome P450 family 2 subfamily C polypeptide 9 [EC:1.14.14.51 1.14.14.52 1.14.14.53 1.14.14.-]	0.0000284	0.0000069
K17721	cytochrome P450 family 2 subfamily C polypeptide 19 [EC:1.14.14.51 1.14.14.52 1.14.14.53 1.14.14.-]	0.0000133	0.0000069
K17726	leukotriene-B4 20-monooxygenase / phylloquinone omega-hydroxylase [EC:1.14.13.30 1.14.13.194]	0.0000000	0.0000502
K18592	gamma-glutamyltranspeptidase / glutathione hydrolase / leukotriene-C4 hydrolase [EC:2.3.2.2 3.4.19.13 3.4.19.14]	0.0000273	0.0000482
K19246	arachidonate 15-lipoxygenase [EC:1.13.11.33]	0.0000000	0.0000007

Supplementary figure legends

Supplementary Figure S1. Comparison of upper airway microbiota between young adults and elderly individuals according to disease status. The significance of differences between groups was tested using Mann-Whitney test. (* $p < 0.05$).

Supplementary Figure S2. Diversity and microbiota were compared according to age in each group.

Supplementary Figure S3. Comparison of diversity and composition of airway microbiota from elderly. (A) Shannon diversity index was compared between non-asthmatic subjects and asthmatic patients after normalization of the read number. (B) The microbiota was compared using the PCoA plot. (C) Phylum composition was compared between the groups. (D) Genus composition was compared between the groups. Frequently detected genera were selected for comparison. The significance of differences between the groups was tested using Mann-Whitney test. (* $p < 0.05$).

Supplementary Figure S4. The relative abundance of airway microbiome pathways from elderly group were clustered in Heatmap analysis. Pathways were selected from differently detected pathway of airway microbiome in young adults.

Supplementary Figure S5. Comparison of diversity and significantly different microbial members according to the severity of asthma in young adults and elderly groups. The severity of asthma was determined by the level of treatment to control symptoms and exacerbations with Global Initiative for Asthma (GINA) step. The significance of differences between groups was tested using the Mann-Whitney test. (* $p < 0.05$).

Supplementary Figure S6. Comparison of functional genes proportions between non-asthmatics and asthmatics in young adults and elderly groups. The higher KEGG Orthology categories (1st and 2nd) were compared. AC: Young adults non-asthmatic controls; AA: Young adults asthmatics; EC: Elderly non-asthmatic controls; EA: Elderly asthmatics. (**p < 0.01).

Figure S1.

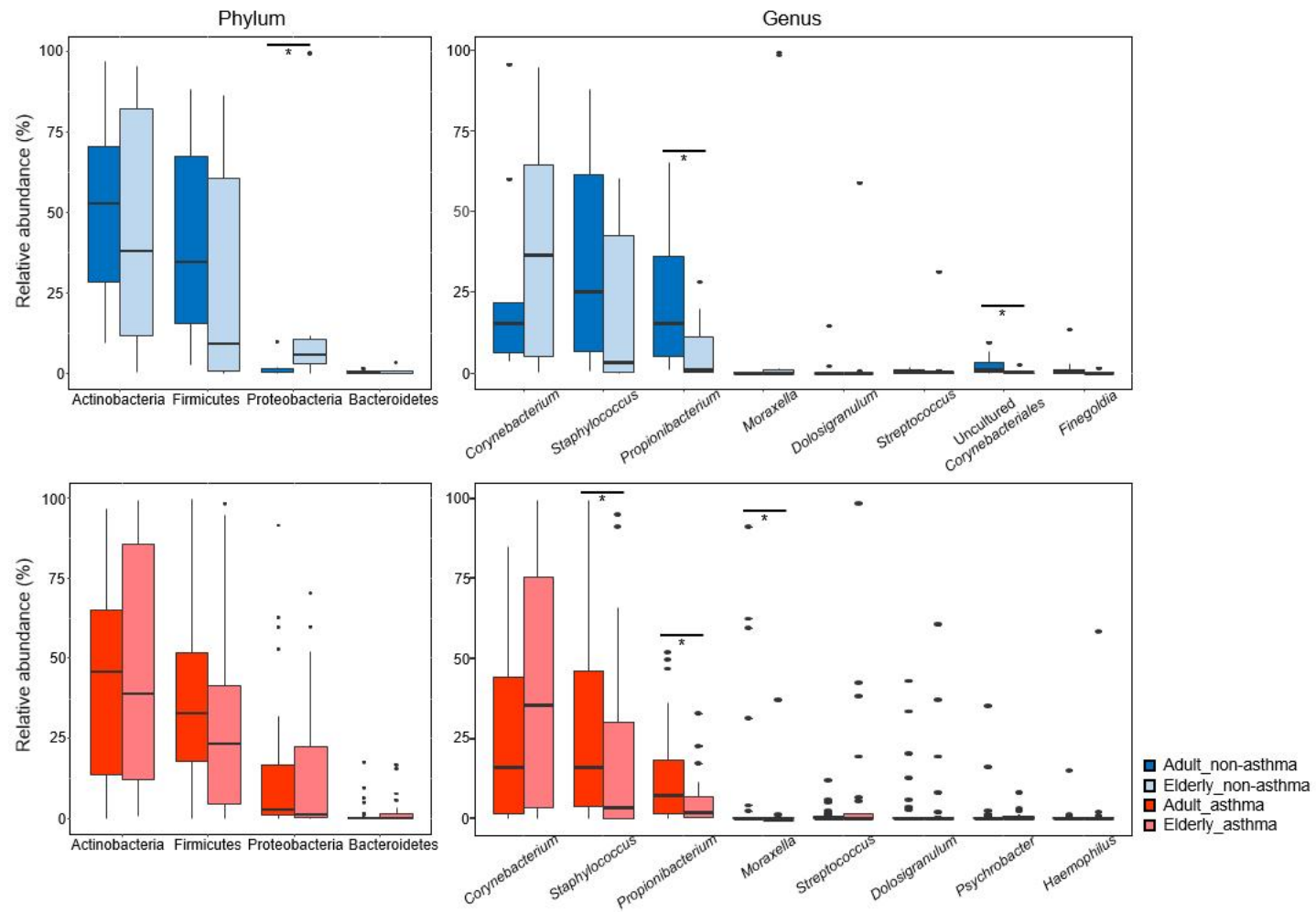


Figure S2.

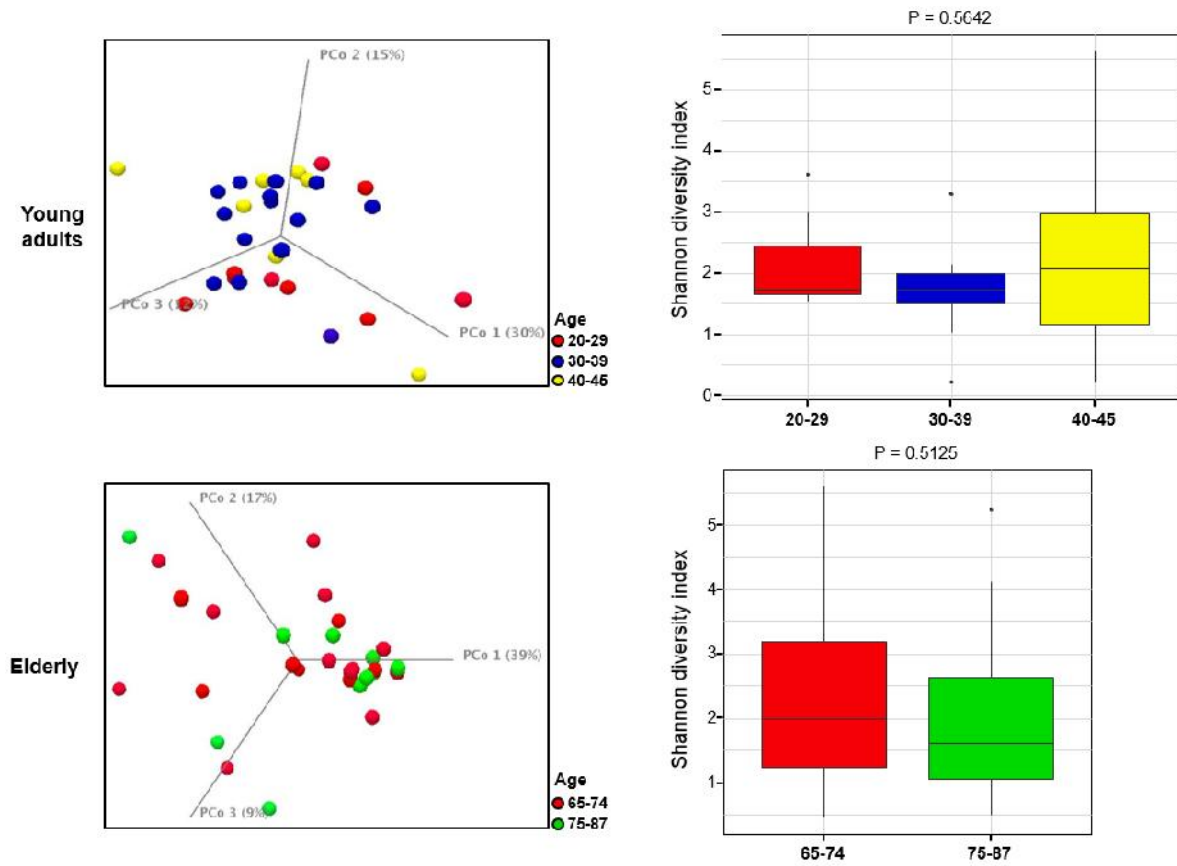


Figure S3.

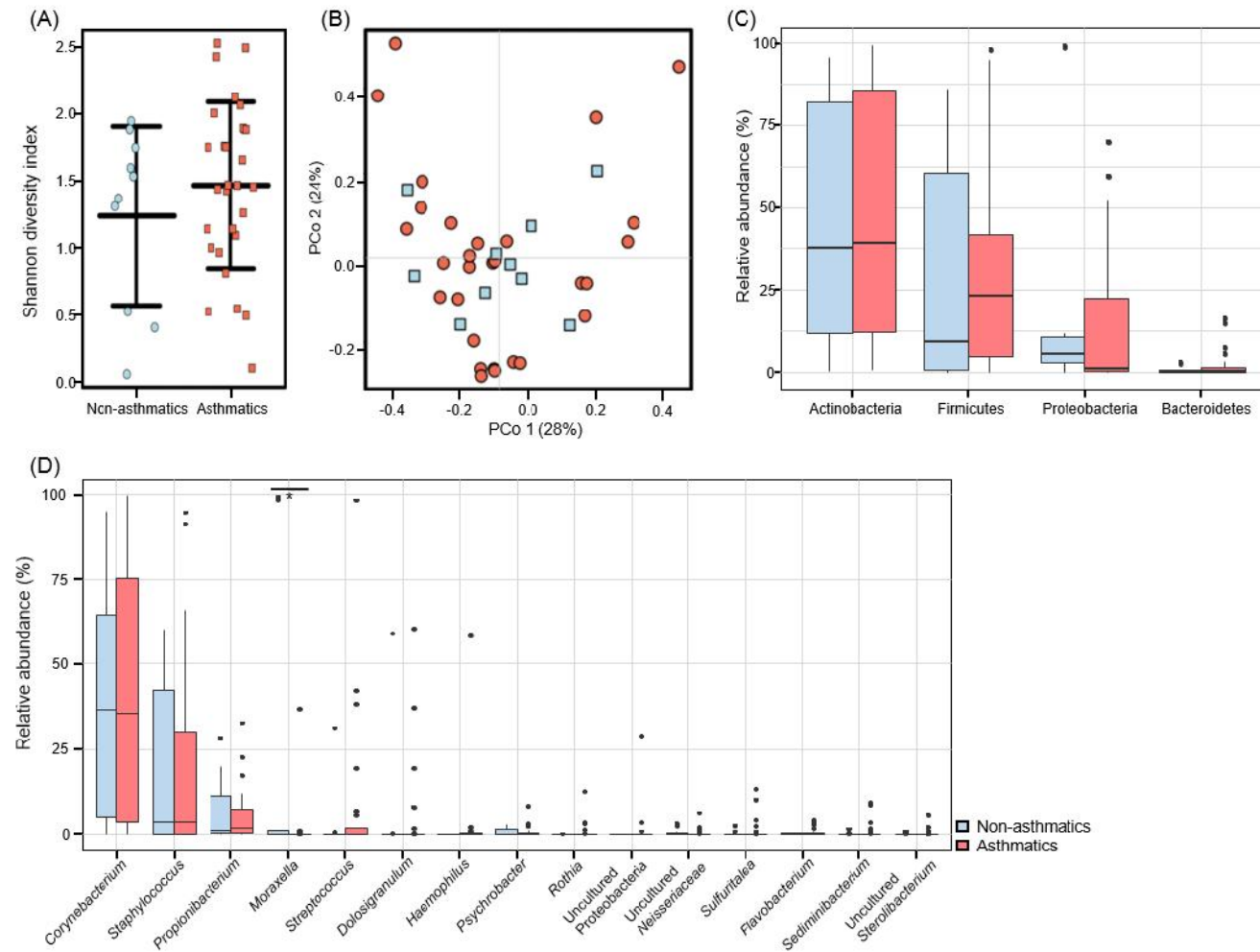


Figure S4.

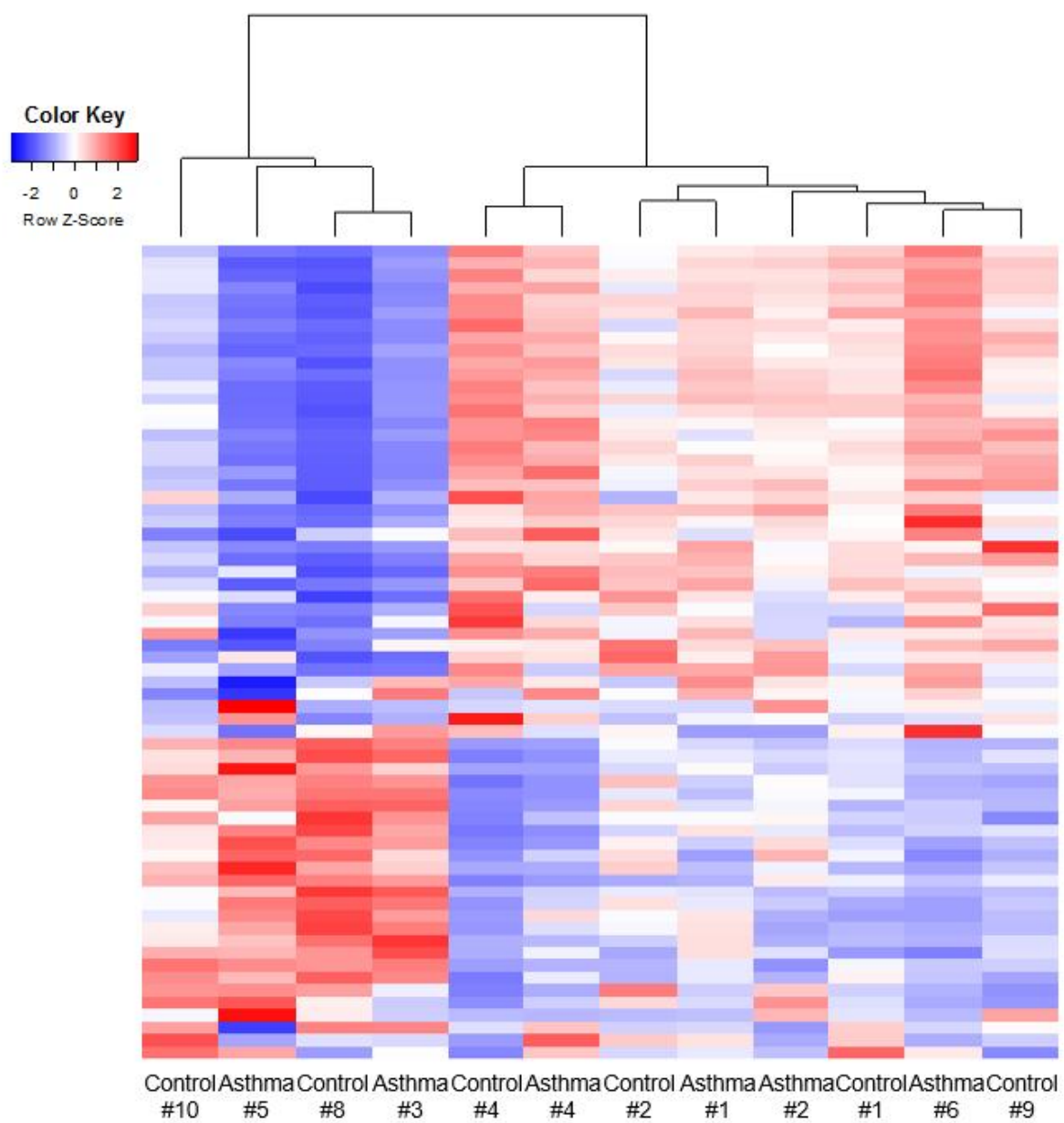


Figure S5.

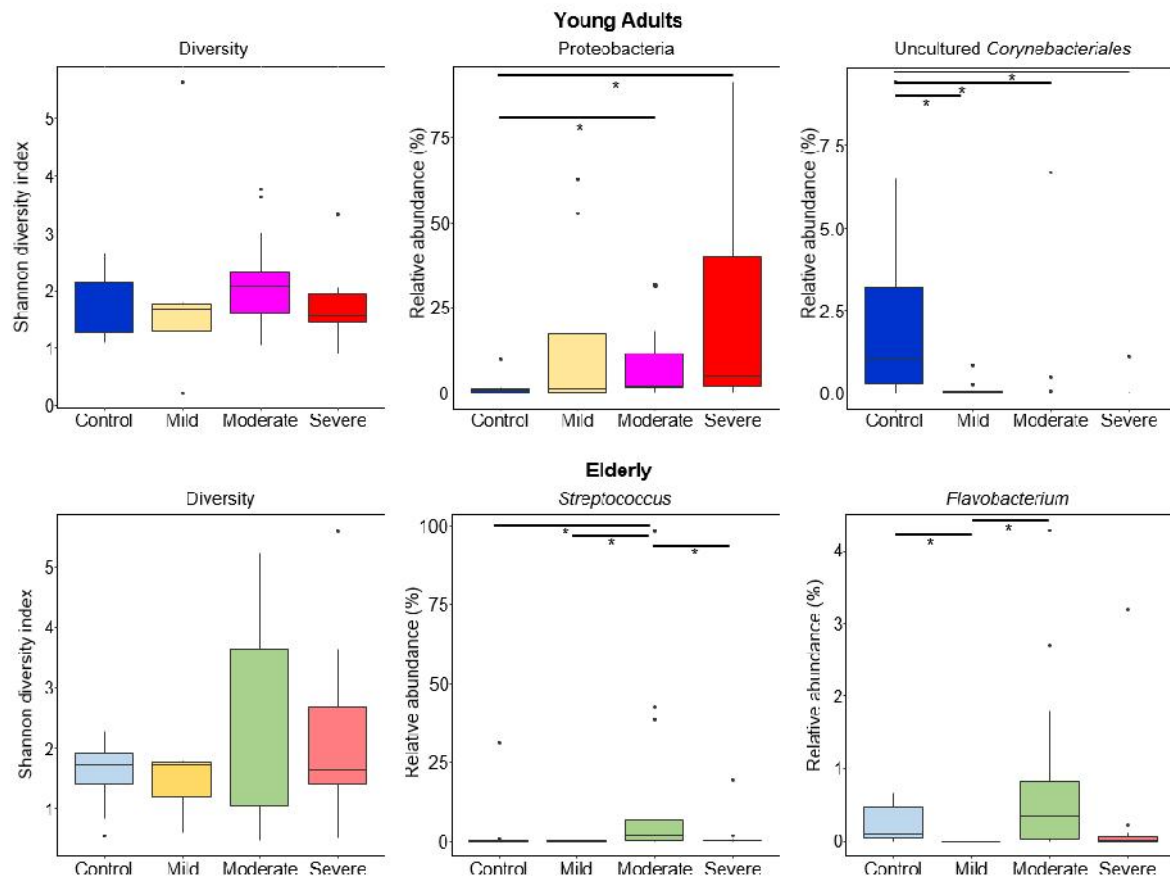


Figure S6.

