



Altered gut microbiome compositions are associated with the severity of asthma

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Background: Despite substantial evidence on the contribution of the diversity of the gut microbiome to the pathogenesis of asthma and allergic diseases, little is known about their relationship with asthma severity and/or clinical phenotypes. We analyzed the difference in composition of the gut microbiome between subjects with asthma and healthy subjects and explored its role in the development of asthma.

Methods: Fecal samples from 15 subjects with severe asthma (SA), 14 with non-severe asthma (NSA), and 15 healthy subjects were assessed by 16S ribosomal RNA gene sequencing methods to identify the gut bacterial composition.

Results: Compared with those in the NSA group, patients in the SA group had a higher dose of inhaled corticosteroids, and there were more atopic subjects (60% vs. 35.7%, respectively). No significant differences were found at the phylum level either in operational taxonomic unit numbers or in diversity scores among the SA, NSA, and healthy groups. However, at the family level, the relative abundance of *Acidaminococcaceae* in the SA group was remarkably lower than that in the group with healthy subjects ($P < 0.05$). Furthermore, *Veillonellaceae* and *Prevotellaceae* were significantly more common in samples from the SA group than in those from the NSA group ($P < 0.05$). In the SA group, positive correlations were observed between the relative abundance of *Veillonellaceae* and mid-expiratory flow 25% (MEF25%) predicted ($r = 0.538$, $P = 0.047$), as well as between the relative abundance of *Acidaminococcaceae* and body mass index ($r = 0.642$, $P = 0.010$). Principal component analysis suggested that the relative abundances of *Acidaminococcaceae* and *Prevotellaceae* were associated with severe asthma. Moreover, we found that class *Betaproteobacteria*, order *Burkholderiales*, and family *Alcaligenaceae* were significantly different among the groups defined by serum immunoglobulin E (IgE) levels.

Conclusions: Our findings suggest that altered gut microbiome compositions are involved in the severity of asthma and that there are specific bacteria related to different asthma phenotypes in terms of serum IgE levels.

Keywords: Gut microbiome; asthma; phenotype

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Introduction

Asthma is a common respiratory disease, which is characterized by chronic inflammation. This condition affects approximately 1–18% of the population in different countries via both complicated etiopathogeneses and unknown pathogeneses (1). Recently, there has been a research focus on microbiota for its potential roles in patients with asthma. This study, as well as other studies, has shown the disordered lung microbiota and its contributions to the severity and phenotypes of asthma (2-7).

In addition to lung microbiota, gut microbiome diversity has been implicated in the pathogenesis of asthma (8-11). The population diversity of intestinal bacteria is closely related to the microenvironment and immune system. Gut microbiota activate immune responses at distal sites (e.g., the lungs) by secreting factors, such as histamine and short-chain fatty acids (SCFAs) (12,13). Early life and eating habits can also affect the population diversity of intestinal bacteria. For example, excess drinks with free fructose probably lead to higher fructose/glucose ratios in the gut, which weakens intestinal absorption of fructose. The variation of microenvironment shifts the diversity of intestinal bacteria, which finally increases the incidence of asthma (14,15). Alterations in the gut microbiota early in life are associated with the risk of asthma (16-23). However, the precise underlying mechanisms of how gut microbiota affect the pathogenesis of asthma remain unknown, especially for severe asthma.

Moreover, it is unknown whether the severity and/or clinical phenotypes of asthma are linked to different profiles of gut microbiota. Therefore, in this study, we assessed 44 fecal samples from patients with and without asthma in the subtropical city of Guangzhou, within Southern China. High-throughput 454 sequencing was utilized to evaluate fecal samples from 15 severe asthma (SA), 14 non-severe asthma (NSA), and 15 healthy subjects.

We present the following article in accordance with the STROBE Checklist (available at <https://dx.doi.org/10.21037/jtd-20-2189>).

Methods

Subjects

In this study, 29 outpatients with physician-diagnosed asthma and 15 healthy subjects were recruited at The First Affiliated Hospital of Guangzhou Medical University between December 2016 and June 2017. In patients with asthma 15 were classified as having SA and 14 were classified as having NSA. The proportions of smokers were similar in both the SA (1/15) and NSA (1/14) groups, and there were no smokers in the healthy group. The diagnoses and severities of asthma were confirmed based on the criteria of the Global Initiative for Asthma (GINA) (1), while severe asthma was defined in accordance with the European Respiratory Society/American Thoracic Society (ERS/ATS) guidelines (24). We grouped these patients with asthma into the following two phenotypes on the basis of total immunoglobulin E (IgE) levels: the high-IgE asthma (HEA; >60 IU/mL) group and the low IgE asthma (LEA; <60 IU/mL) group. The exclusion criteria considered for this study included the following: subjects diagnosed with other lung diseases (chronic obstructive pulmonary disease, bronchiectasis and interstitial pneumonia), autoimmune diseases (inflammatory bowel disease), or other diseases known to affect the diagnosis of asthma. Patients taking antibiotics at the time of this study or those receiving immunosuppressive therapies and/or allergen-specific immunotherapies within the last year were also excluded from the study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all subjects and the research was approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (approval No.2016-68).

Atopy

Levels of specific IgE to common aeroallergens (Hx2 and Mx2) and the food allergen (Fx5) were determined using the

ImmunoCAP system (Thermo Scientific, Uppsala, Sweden). Specific IgE was defined as positive if the measurement was ≥ 0.35 IU/mL. Subjects were characterized as atopic if they had at least one positive specific IgE.

Collection and processing of fecal samples

Fresh fecal samples (1–2 g per person) were collected and transferred from bedpans that had been disinfected with 75% ethanol. The fecal samples were then manipulated and homogenized. The prepared samples were then collected and stored at -80°C until further use (11).

DNA extraction and sequencing

The fecal samples (10–50 g) were collected with Tris-EDTA buffer (10 mM Tris-HCl, 1 M EDTA, pH 8.0). Bacterial DNA was isolated and purified using a NEBnext microbiome DNA enrichment kit (New England Biolabs, Ipswich, MA, USA) in line with the manufacturer's instructions. A standard concentration of 10 ng/mL of DNA was prepared for each individual sample for all PCR assays. After performing a sample quality test, we used those quantified DNA samples to construct a library. The bacterial DNA was amplified for the V4 hypervariable region of bacterial 16s rRNA. PCR was amplified with the fusion primer; then, short fragments were removed using Ampure beads. Finally, the quantified library was used for sequencing with the Illumina Miseq 2500 platform (BGI, Shenzhen, China).

Statistical analysis

Reads that did not match the barcode or the primer sequences were excluded before data analysis. Unique reads were pre-clustered by scripts written in Mothur (version 1.31.2, <http://www.mothur.org/>), within two mismatch similarities. Operational taxonomic unit (OTU) numbers were assessed by scripts written in Mothur based on 97% similarities. First, unique reads were aligned to the Ribosome Database Project database using scripts of software written in Mothur and were subsequently annotated. Furthermore, when more than 51% of unique reads of the OTUs were interpreted to belong to one species, we defined these OTUs as representing a unique species. Then, OTUs were calculated by taxonomic analysis at a higher level, such as from the genus level, to the family level, and were summarized using Mothur software. To determine abundance information of the fecal samples,

the numbers of reads in each OTU were computed. The distribution of all annotated species or OTUs at different taxonomic levels (i.e., phylum, class, order, family, and genus) among different samples was analyzed and summarized by scripts of software written in QIIME (version 1.50). We not only used the Chao1 estimator and ACE to estimate species richness but also used Shannon-Wiener and Simpson indices to assess the ecological diversity of various species. Differences in bacterial abundances and clinical parameters between healthy and asthmatic subjects were assessed using analysis of variance (ANOVA). We performed linear correlations to determine associations among clinical and demographical features and bacterial abundances in healthy subjects and patients with asthma. We selected 30 variables in cluster analysis; 22 variables were excluded as being clinically irrelevant for the clustering and being represented by other variables. Eventually, eight variables were selected. The SPSS statistical software package (version 18.0) was used for all statistical analyses. A P value < 0.05 was considered to be statistically significant. Data were reported as the mean \pm standard deviation or median with interquartile range. Analysis of LSD, Bonferroni, and Spearman's rank correlation coefficient was used for parametric continuous data; analysis of Kruskal-Wallis, Mann-Whitney U, and Pearson's correlation was used for nonparametric continuous data; and the chi-square test was used for categorical variables.

Results

Sociodemographic characteristics of the included subjects

Sociodemographic and clinical features of the subjects are presented in *Table 1*. There were no significant differences in gender or body mass index (BMI) among the three groups: patients with SA, referred as the SA group, patients with NSA, referred as the NSA group, and the healthy subjects, referred as the healthy group, in this article. Compared with the healthy group, the SA and NSA groups showed higher eosinophils in both sputum and blood, suggestive of eosinophilic-predominant airway inflammation. Compared with the NSA group, the SA group had a higher dose of inhaled corticosteroids (ICS) and a higher percentage of atopy (60% vs. 35.7%, respectively; $P < 0.05$).

Compositions of gut microbiota

We assessed bacterial richness by OTU numbers and

Table 1 Sociodemographic characteristics of subjects determined by the degree of clinical symptoms

Characteristics	Severe asthma group	Non-severe asthma group	Healthy group	P value
Sample size (%)	15 (34.1)	14 (31.8)	15 (34.1)	–
Female (%)	9 (60.0)	8 (57.1)	8 (53.3)	0.76
BMI (kg/m ²)	24.5±0.7	24.2±0.7	19.8±2.4	0.105
Age (years)	49±3.7	55±3.5	52±7.3	0.027 [#]
Asthma duration (years)	9.8 (2, 17.6)	12.2 (5.8, 18.6)	–	0.093
Acute attacks (times/year)	0 (0.2, 1.8)	0.5 (0.2, 0.9)	–	0.852
Nasal disease duration (years)	8.5 (0.6, 16.4)	13.1 (6.2, 20)	–	0.364
OCS (sample size, %)				
0 mg	10 (66.7)	11 (78.6)	–	0.602
0–10 mg	2 (20.0)	1 (7.1)	–	0.602
>10 mg	3 (13.3)	2 (14.3)	–	0.602
[§] ICS	3 (2.9, 3)	2.6 (2.4, 2.6)	–	<0.001 [§]
Sputum eosinophils (%)	17.6±5.6	6.8±5.7	1.5±1	<0.001 ^{*#}
Sputum neutrophils (%)	46.3±6.5	65.5±9.5	68±8.3	0.208
ACT score	23±2.4	23±0.3	–	0.216
FVC%-predicted (%)	88.3±4	90±6.5	92±3.8	0.406
FEV1%-predicted (%)	72.7±6.1	81±6.9	94±5.4	<0.001 ^{*#}
FEV1/FVC ratio	69.3±2.7	67.9±2.6	87.2±3.3	<0.001 ^{*#}
PEF%-predicted (%)	78.4±5.7	77±9.2	101±8	0.009 [#]
FEF25–75%-predicted (%)	19.3±5.2	35.5±5.1	81.5±4.5	<0.001 ^{*#}
MEF75%-predicted (%)	55.6±6.8	47±9.9	94±12.6	0.002 ^{*#}
MEF50%-predicted (%)	38.8±5.8	41.5±5.7	78±10.9	<0.001 ^{*#}
MEF25%-predicted (%)	26.7±5	42±5.2	82±6.4	<0.001 ^{*#&}
Blood eosinophils (%)	4.6±1.3	3.4±0.5	1.6±0.2	<0.001 ^{*#}
[§] Total IgE (IU/mL)	7.±0.6	7.5±0.5	4.6±0.5	<0.001 ^{*#}
Atopy (%)	9 (60.0)	5 (35.7)	0	0.048 ^{&}

^{*}, severe asthma (SA) group vs. healthy group; [#], non-severe asthma (NSA) group vs. Healthy group; [&], SA group vs. NSA group; Inhaled corticosteroid (ICS) was normalized to budesonide. [§], logarithmic transformation was used because data of Inhaled corticosteroid (ICS) dose and serum total IgE presented skewed distribution. Data expressed as mean ± standard deviation, or median (interquartile range), or percentage (%). P values were determined using *t* test, Mann-Whitney U test, or chi-square test among two groups and by using LSD, Bonferroni, and Kruskal-Wallis tests among three groups. ICS, inhale corticosteroid, OCS, oral corticosteroid, BMI, body mass index, FVC%-pred, forced vital capacity per predicted, FEV1%-predicted: Forced expired volume in one second per predicted, FEV1/FVC ratio, forced expiratory volume in one second to forced vital capacity ratio, PEF%-predicted, peak expiratory flow rate per predicted, FEF25–75%-predicted, force expiratory flow from 25–75% of FVC per predicted, MEF75%-predicted, maximal expiratory flow after 75% of the FVC per predicted, MEF50%-predicted, maximal expiratory flow after 50% of the FVC per predicted, MEF25%-predicted, maximal expiratory flow after 25% of the FVC per predicted.

evaluated species diversity by Chao, ACE, Shannon, and Simpson indices. In this study, a total of 3,274,052 raw reads were generated for all samples, of which 2,651,837 quality sequence reads were used for subsequent OTU analyses. We found no statistically significant differences among the three groups in the number of OTU, Chao, ACE, Shannon, or Simpson indices, indicating that gut microbial richness and diversity were quite equal among the three groups. We also identified 11 bacterial phyla in all of the fecal samples, of which *Bacteroidetes*, *Proteobacteria*, *Firmicutes*, and *Fusobacteria* contributed to over 90% of the total sequences in all three groups (Figure 1). There was no significant difference in the relative abundance of bacteria at the phylum level among the three groups. However, at the family level, we confirmed that the relative abundance of *Acidaminococcaceae* was remarkably lower in the SA group, than that in the healthy subjects ($P < 0.05$). *Lachnospiraceae*

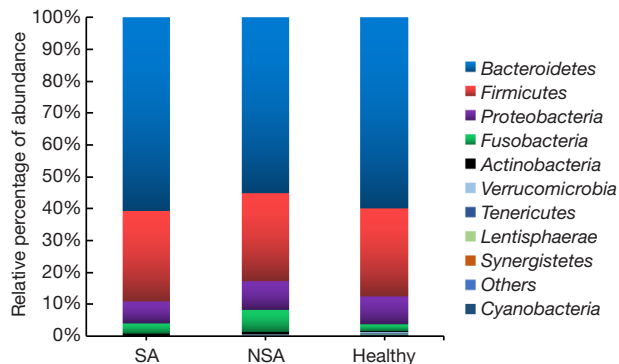


Figure 1 Stacked bar chart showing bacterial composition (median values) at the phylum level in the subjects with severe asthma (SA), subjects with non-severe asthma (NSA), and healthy subjects. *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, and *Proteobacteria* contributed over 90% of the total sequences in all three groups.

had a higher proportion in the NSA group than in the healthy group ($P < 0.05$). *Veillonellaceae* and *Prevotellaceae* were observably more common in samples from the SA group than those from the NSA group ($P < 0.05$) (Table 2 and Figure 2).

The association of gut microbiota and severity of asthma

Analysis based on the family level showed that the relative abundance of *Veillonellaceae* was positively related to the proportion of sputum eosinophilia in the NSA group ($r = 0.579$, $P = 0.030$) (Figure 3A). In contrast, the relative abundance of *Lachnospiraceae* was negatively correlated with sputum neutrophils (%) in the NSA group ($r = -0.635$, $P = 0.015$) (Figure 3B). Additionally, positive associations were observed not only between the relative abundance of *Veillonellaceae* and MEF25% (Figure 3C), but also between that of *Acidaminococcaceae* and BMI ($r = 0.642$, $P = 0.010$) (Figure 3D) in the SA group.

Differential gut microbiome profiles according to the level of total IgE

In view of the potential role of total IgE levels in the gut microbiome profiles, we grouped our patients into the high-IgE asthma (HEA) group, low-IgE asthma (LEA) group, and healthy group. As shown in Table 3, there were 21 subjects (72.4% of all of the asthmatic patients) in the HEA group. Among them, 11 cases belonged to the SA group and 10 cases to the NSA group. Compared with the LEA group, acute attacks were obviously elevated in the HEA group. No statistical differences in OTU numbers, and Chao, ACE, Shannon, and Simpson indices were observed among the three groups. Similar to the SA and NSA groups, a core gut microbiome consisting of *Bacteroidetes*,

Table 2 Relative abundances of different bacteria at the family level

Level	Bacterial name	Median value			P value		
		SA	NSA	Healthy	SA vs. NSA	SA vs. Healthy	NSA vs. Healthy
Family	<i>Prevotellaceae</i>	0.228 (0.056, 0.400)	0.004 (-0.002, 0.010)	0.156 (0.010, 0.301)	0.016	0.558	0.416
	<i>Veillonellaceae</i>	0.066 (-0.004, 0.135)	0.011 (-0.002, 0.023)	0.020 (-0.002, 0.043)	0.023	0.060	1
	<i>Acidaminococcaceae</i>	0.024 (-0.007, 0.055)	0.026 (0.015, 0.037)	0.044 (0.018, 0.069)	0.311	0.022	0.934
	<i>Lachnospiraceae</i>	0.099 (0.065, 0.133)	0.143 (0.111, 0.175)	0.060 (0.029, 0.092)	0.082	1	0.024

SA, severe asthma; NSA, non-severe asthma; P values were determined by Spearman's tests.

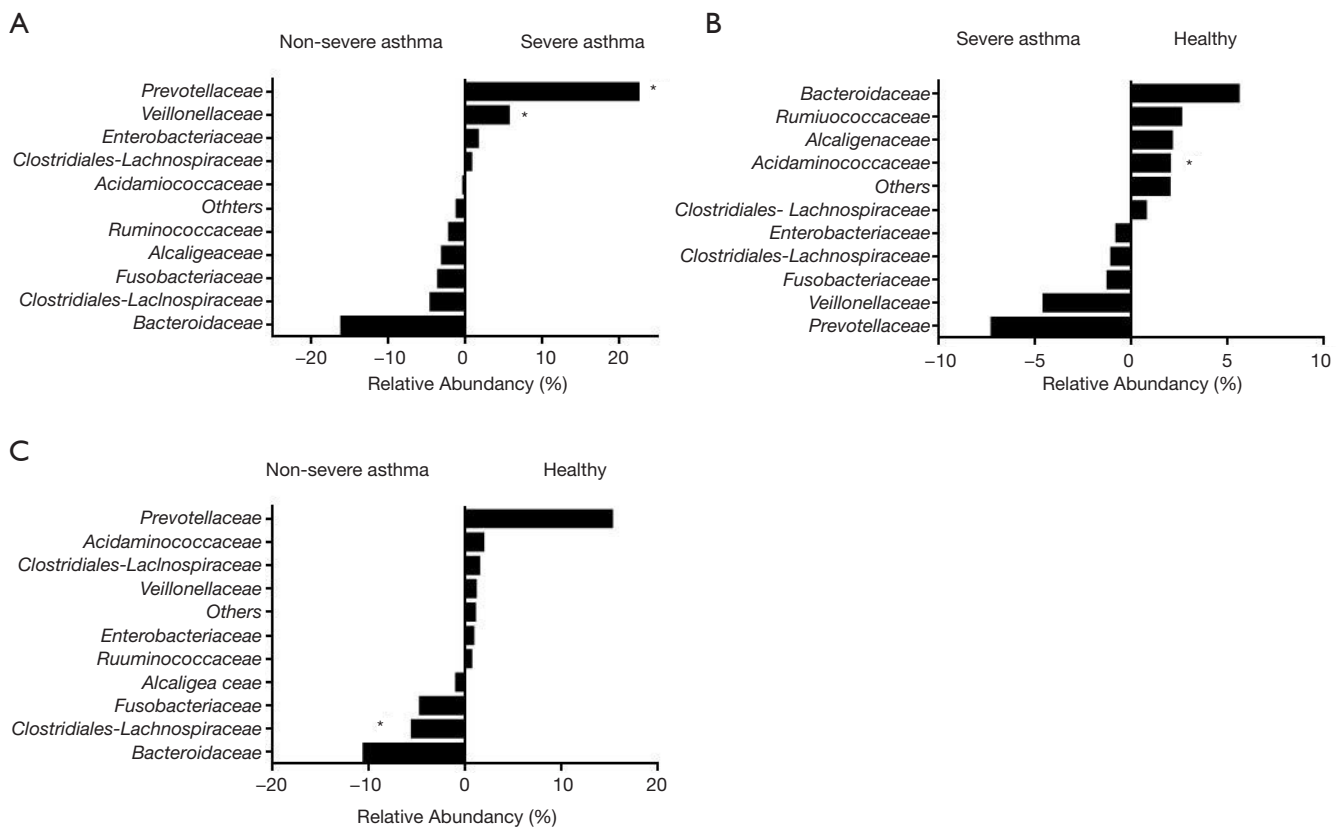


Figure 2 Correlations of relative abundances of bacteria among the non-severe asthma (NSA) and severe asthma (SA) groups (A), healthy and severe asthma (SA) groups (B), and healthy and non-severe asthma (NSA) groups (C). The value of zero indicates a similar percentage contribution of the bacterial family between the groups. A positive value for a bacterial family indicates higher percentage abundance in the group (right side), and a negative value indicates higher abundance in the group (left side). * indicates the statistical significance between two groups in the relative abundance of bacteria (Mann-Whitney U test, $P < 0.05$).

Proteobacteria, *Firmicutes*, and *Fusobacteria* at the phylum level was identified in the HEA and LEA groups (Figure 4). No significant differences in the relative abundance of bacteria at the phylum level were detected among the three groups. However, at the class level, *Betaproteobacteria* was more frequent in the LEA group than in the HEA group ($P = 0.018$). Moreover, *Burkholderiales* was significantly more abundant in the LEA group than in the HEA group at the order level ($P = 0.018$). Finally, *Alcaligenaceae* was confirmed to be more common in the LEA group than in the HEA group at the family level ($P = 0.019$) (Table 4, Figure 5).

According to correlation analysis, nasal disease duration and serum total IgE showed a positive correlation between the three bacteria levels (*Betaproteobacteria* class, *Burkholderiales* order, and *Alcaligenaceae* family) in the HEA group ($P < 0.05$) (Figure 6A I-VI). In contrast, significant negative correlations were found between the

relative abundance of the abovementioned bacteria and the dose of oral steroids and sputum eosinophilia in the HEA group (Figure 6B I-VI). The relative abundance of *Betaproteobacteria* class, *Burkholderiales* order, and *Alcaligenaceae* family showed a positive relationship with the blood eosinophilic (%) ($P < 0.05$) in the LEA group (Figure 6C I-III).

Principal component analysis

Five principal components (PC1-5) with eight values > 0.8 and accounting for $> 10\%$ of the variance were identified (Table 5). PC1 was characterized by severe asthma, which correlated with a worse reaction to ICS and an elevation of sputum neutrophils. Moreover, PC1 was associated with an increased abundance of *Acidaminococcaceae*. The features of PC2 included longer duration of asthma and nasosinusitis,

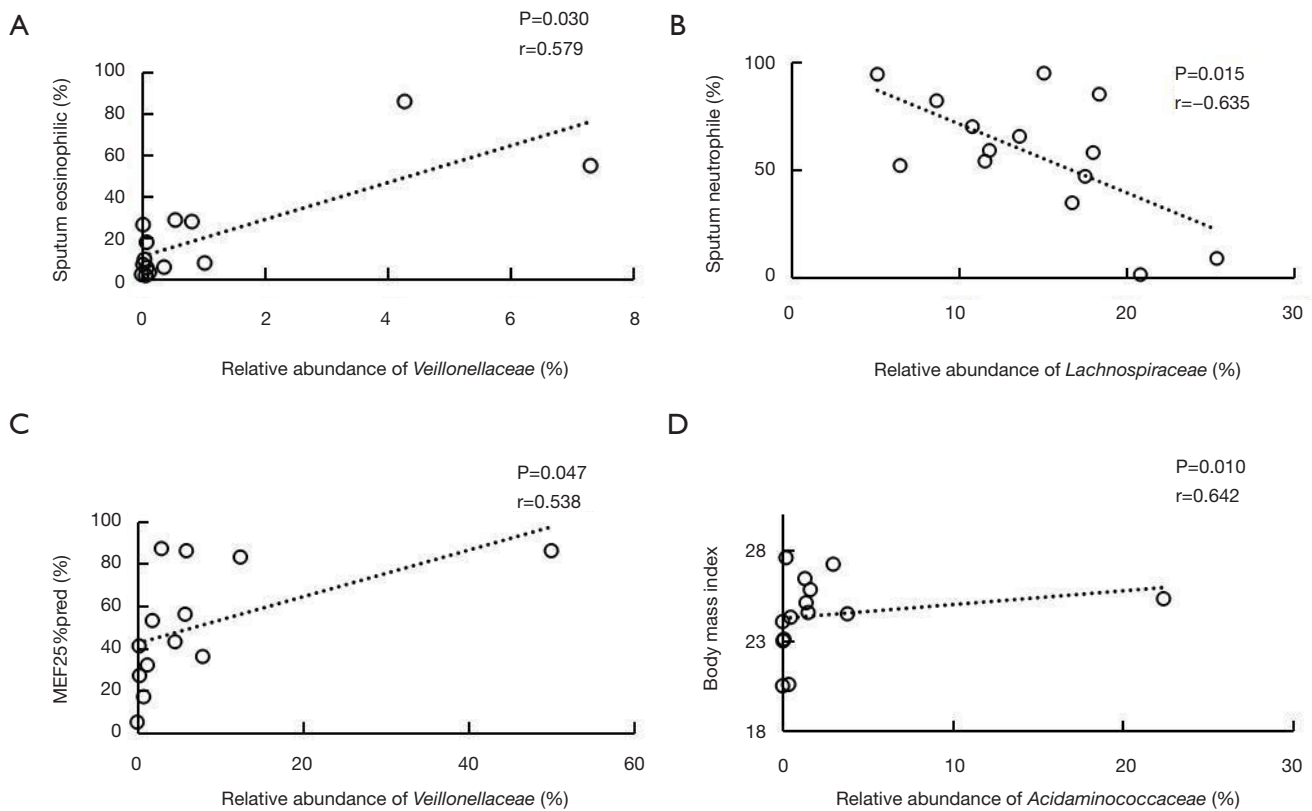


Figure 3 Scatter plots and correlations. (A) Scatter plot of eosinophil count (%) in induced sputum and relative of *Veillonellaceae* in fecal samples of subjects from the non-severe asthma (NSA) group. (B) Scatter plot of neutrophil count (%) in induced sputum and relative abundance of *Lachnospiraceae* in fecal samples of subjects from the NSA group. (C) Scatter plot of mid-expiratory flow 25% (MEF25%) predicted (%) and relative abundance of *Veillonellaceae* in fecal samples of subjects from the severe asthma (SA) group. (D) Scatter plots of body mass index and relative abundance of *Acidaminococcaceae* in fecal samples of subjects from the SA group (Spearman's rank correlation coefficient, $P < 0.05$).

without associated bacterial species. PC3, which had a lower abundance of *Prevotellaceae*, was also related to severe asthma. PC4 was grouped into non-severe asthma, with a better reaction to ICS and an increased abundance of *Veillonellaceae*. PC5 was typified by upregulated sputum neutrophils, reduction of *Prevotellaceae* abundance, and elevation of *Acidaminococcaceae* abundance.

Discussion

Previous studies have shown that intestinal bacteria are closely related to the occurrence and development of asthma in infants and adults (8,11,18,25-29). To our knowledge, a few studies have reported on the relationships of gut microbiota with different levels of severity and serum total IgE in asthma.

This study collected 44 fecal samples (29 subjects with asthma and 15 healthy) to conduct DNA sequencing. Twenty-nine subjects with asthma were grouped into two subgroup based on the inhaled corticosteroid (ICS) dose and Total IgE (Tables 1 and 4). The aim of this study was to explore the relationship between the characteristics of intestinal flora from patients with asthma and asthmatic phenotype.

The diagnosis of severe and non-severe asthma was established based on the criteria of the GINA (1), and European Respiratory Society/American Thoracic Society (ERS/ATS) guidelines (24). Our results showed that four predominate bacterial phyla exist in the gut among the SA, NSA and healthy groups. They included *Bacteroidetes*, *Proteobacteria*, *Firmicutes*, and *Fusobacteria* (Figure 1). This result was inconsistent with the findings of Hevia *et al.* (11).

Table 3 Demographic and clinical characteristics of the recruited subjects classified by serum IgE levels

Characteristics	High-IgE group	Low-IgE group	Healthy group	P value
Sample (%)	21 (47.7)	8 (18.2)	15 (34.1)	–
Female (%)	14 (66.7)	3 (37.5)	8 (53.3)	0.218
BMI (kg/m ²)	23.3±0.5	24.3±0.8	22.5±1.1	0.202
Asthma duration (years)	9.5 (5.1, 13.8)	12.8 (3.3, 22.2)	–	0.518
Acute attacks (times/years)	1.1 (0.5, 1.7)	0	–	0.005 [§]
Nasal disease duration (years)	9.3 (5,13.6)	8.9 (0.1, 17.6)	–	0.549
OCS (sample size, %)	7 (33.3)	0 (0)	–	0.142
[§] ICS	3.2 (2.5, 3.4)	2.9 (2.8, 3.1)	–	0.401
Sputum eosinophils (%)	23.8±4.5	11.2±6.3	1.2±0.5	<0.001*
Sputum neutrophils (%)	53.4±4.9	36.4±11	55.9±8.9	0.305
ACT score	22.7±0.3	23.1±0.2	–	0.684
FVC%-predicted (%)	88.1±4	92±4.1	96.8±2.8	0.400
FEV1%-predicted (%)	72±5	72±5.3	95.9±2.9	0.004* [#]
FEV1/FVC ratio	66.4±2.5	65.3±4.1	84.3±2.3	<0.001* [#]
PEF%-predicted (%)	75.6±5.5	83.2±9	96.8±5.8	0.09
FEF25-75%-predicted (%)	36±4.6	35±5.4	79.7±5.8	<0.001* [#]
MEF75%-predicted (%)	55±7.1	43±8	85±9.3	0.010 [#]
MEF50%-predicted (%)	39.4±5.1	41.1±7.4	80.4±6	<0.001* [#]
MEF25%-predicted (%)	36±4.3	30.5±3.8	75.4±4	<0.001* [#]
Blood eosinophils (%)	5.6±0.8	3.3±0.4	1.3±0.2	<0.001*
[§] Total IgE (IU/mL)	2.4 (2.3, 2.7)	1.6 (1, 1.8)	1.3 (0.9, 1.5)	<0.001* [§]

*, high-IgE asthma (HEA) group vs. healthy group; #, low-IgE asthma (LEA) group vs. healthy group; §, HEA group vs. LEA group, Inhaled corticosteroid (ICS) was normalized to budesonide; §, logarithmic transformation was used because ICS dose and serum Total IgE data presented skewed distribution. Data expressed as mean ± standard deviation, or median (interquartile range), or percentage (%). P values were determined using *t* test, Mann-Whitney U test, or chi-square test among two groups and by using LSD, Bonferroni, and Kruskal-Wallis tests among three groups. ICS, inhaled corticosteroid, OCS, oral corticosteroid, BMI, body mass index, FVC%-pred, forced vital capacity per predicted, FEV1%-predicted: Forced expired volume in one second per predicted, FEV1/FVC ratio, forced expiratory volume in one second to forced vital capacity ratio, PEF%-predicted, peak expiratory flow rate per predicted, FEF25–75%-predicted, force expiratory flow from 25–75% of FVC per predicted, MEF75%-predicted, maximal expiratory flow after 75% of the FVC per predicted, MEF50%-predicted, maximal expiratory flow after 50% of the FVC per predicted, MEF25%-predicted, maximal expiratory flow after 25% of the FVC per predicted.

and Hua *et al.* (30). We did not obtain significant alpha-diversity indexes, including Shannon, Simpson, Chao1, and ACE indices, among the SA, NSA, and healthy individuals.

A recent study has suggested that higher relative abundances of airborne *Lachnospiraceae* and *Prevotellaceae* lower the prevalence of asthma and allergy (26). Moreover, lower *Lachnospiraceae* in a three-month infant was identified as a risk factor of an early allergic wheezy phenotype, whereas *Veillonellaceae* was a protective factor against allergic

disease (27). However, in our present study, *Lachnospiraceae*, *Veillonellaceae* and *Prevotellaceae* families were more common in the guts of individuals in the SA and NSA groups (Table 2, Figure 2). We adopted different ways of classification according to the levels of severity of asthma and Total IgE, which caused discrepancy in previous studies. Furthermore, the mode of delivery, antibiotics given to the mother or infant, the mode of infant feeding, environment (31,32), food habits, gene differences (33), and anatomical and

physiochemical differences among the intestines (34) influence the establishment of the gut microbiota. The subjects of this study were Chinese, whose environment, food habits, and genes were different from those in European and American subjects. Therefore, the results of this study are helpful to explore the relationship between the diversity of intestinal flora and the development of asthma in different regions of the world.

A previous study suggested that two strains of *Veillonella* in stool samples of atopic wheezing children (22). Additionally, Arrieta *et al.* have found that intestinal *Prevotellaceae* family induces TH2 immune responses (35), which was probably closely related to asthma. Furthermore, some studies have indicated that *Lachnospiraceae* family play an important role in remodeling of airways (36), which was also likely associated with obstructive ventilation dysfunction in asthma patients. The major modulation of intestinal

flora on the disease may involve the following mechanisms: First, the molecular structure of MAMPs (including LPS and polysaccharide A) for intestinal flora is recognized by pattern-recognition receptors in humans and activate the nuclear factor κ B pathway, thus inducing transcription of proinflammatory and other cytokine genes (37,38). Second, SCFAs, including acetate, propionate, and butyrate, the major end-products metabolized by mainly anaerobic bacteria in the intestine, affect the colonization of other bacteria in the intestine and contribute to maintaining the intestinal epithelium, allergen uptake, and immunological responses in allergic asthma (39). *Prevotellaceae* family has the structure of MAMPs (LPS) (40,41), and it has the ability to produce SCFAs, as do the *Veillonellaceae* family, *Acidaminococcaceae* family, and *Lachnospiraceae* family (40,41). We speculated that the cellular structure and metabolites of the intestinal flora may be related to asthma severity levels.

To further explore the relationship between different intestinal bacteria and asthma, we took the data of OTUs from intestinal flora and clinical indicators to make the correlation analysis. The relative abundance of *Veillonellaceae* showed a positive correlation with MEF25% predicted and sputum eosinophils (%) in the SA and NSA groups (Figure 3A,C). *Veillonella* takes part in atopic wheezing and airway obstructions in children (35) and adults (28). It populates the small intestine and appears to block IL-12p70 production while augmenting IL-8, IL-6, IL-10, and TNF- α responses by dendritic cells in *in vitro* assays; indeed, these cytokines have been linked to airway obstruction and severe asthma phenotype (41-45), as well as promotion of eosinophil recruitment and airway inflammation (46-48). A growing number of studies have found that the increase of the relative abundance of *Lachnospiraceae* family inhibits the production of IL-17, the cytokine that may lead to improvement in clinical symptoms of asthma (36,49), and indirectly results in the reduction of neutrophilic ratio in

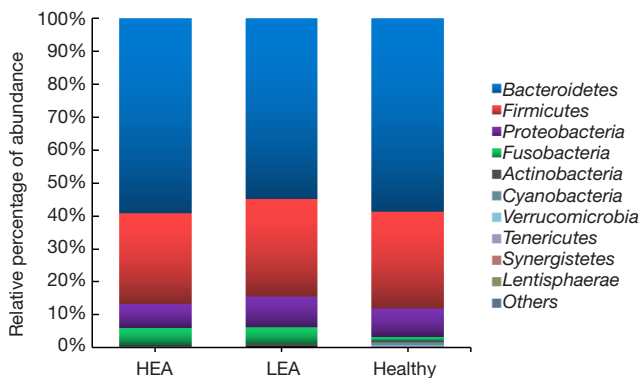


Figure 4 Stacked bar chart showing bacterial composition (median values) at the phylum level in the high-immunoglobulin E (IgE) asthma subjects (HEA), low-IgE asthma subjects (LEA), and healthy subjects. A core gut microbiome consisting of *Bacteroidetes*, *Firmicutes*, *Fusobacteria*, and *Proteobacteria* was identified in all three groups.

Table 4 Relative abundances of different bacteria at the family level

Level	Bacteria name	Median value			P value		
		HEA	LEA	Healthy	HEA vs. LEA	HEA vs. Healthy	LEA vs. Healthy
Class	<i>Betaproteobacteria</i>	0.032 (0.018, 0.045)	0.076 (0.034, 0.119)	0.047 (0.029, 0.066)	0.018	0.556	0.481
Order	<i>Burkholderiales</i>	0.032 (0.018, 0.045)	0.076 (0.034, 0.119)	0.047 (0.029, 0.066)	0.018	0.556	0.481
Family	<i>Acidaminococcaceae</i>	0.029 (0.007, 0.051)	0.014 (0, 0.027)	0.05 (0.017, 0.083)	1	0.093	0.050
	<i>Alcaligenaceae</i>	0.031 (0.018, 0.045)	0.076 (0.033, 0.119)	0.047 (0.029, 0.065)	0.019	0.536	0.518

HEA, high IgE group; LEA, low IgE group; P values were determined using Spearman's tests.

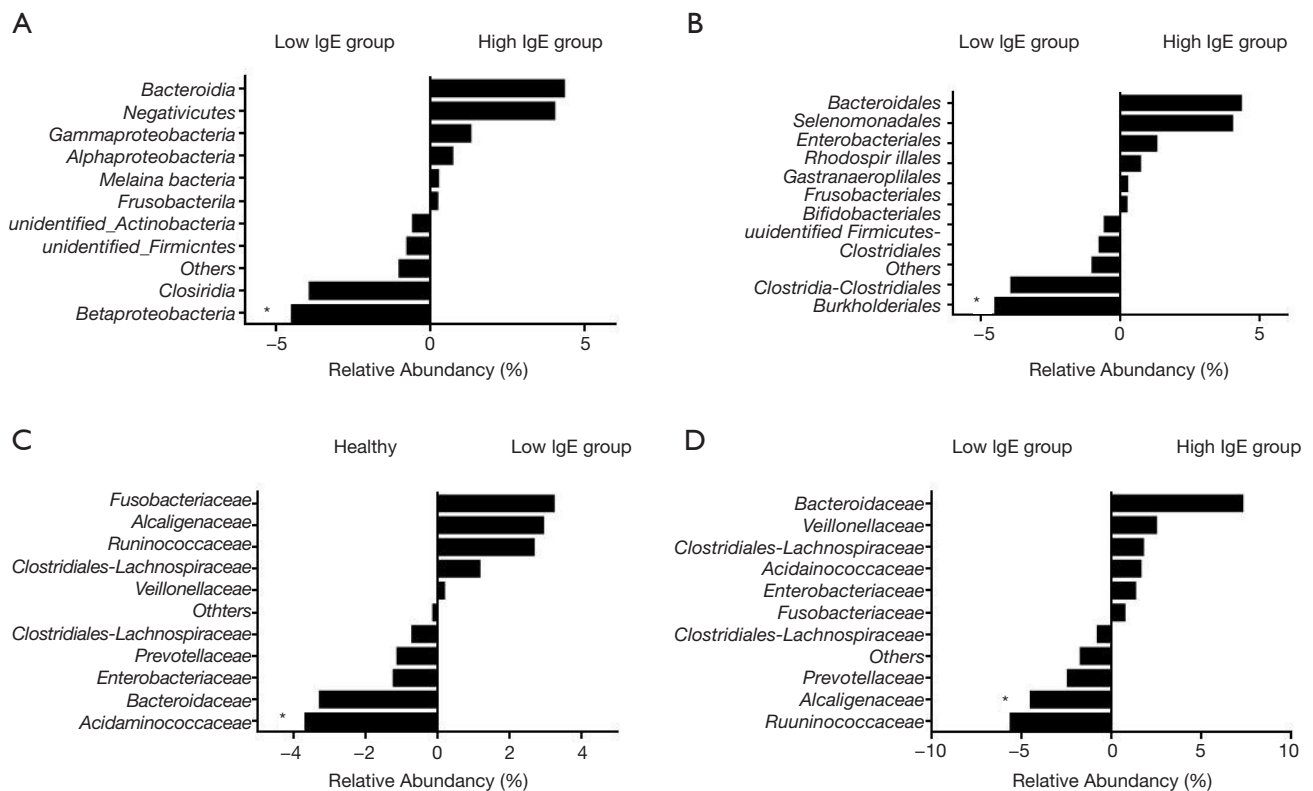
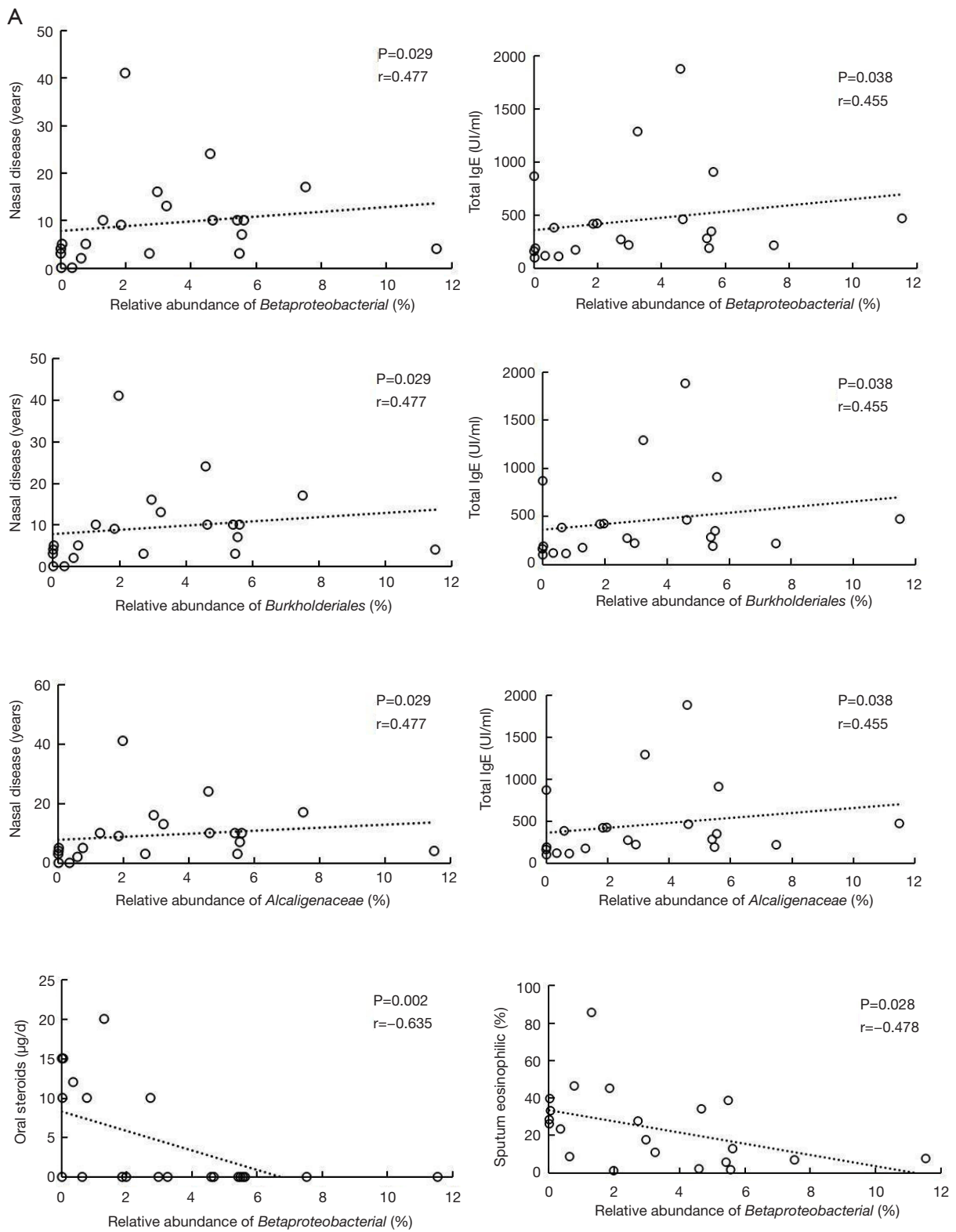


Figure 5 Correlations of relative abundances of bacteria among low-IgE asthma groups (LEA) and high-IgE asthma (HEA) groups (A,B,D), low-IgE asthma (LEA) groups and the healthy group (C). The value of zero indicates a similar percentage contribution of the bacterial family in both groups. A positive value for a bacterial family represents higher percentage abundance in the group on the right, and a negative value indicates higher abundance in the group on the left. * indicates statistically significant difference in the relative abundance of bacteria between the two groups (Mann-Whitney U test, $P < 0.05$).

induced sputum (50). This was similar to the results of our study (Figure 3B). Finally, as an opportunistic pathogen of the intestine, genus *Acidaminococcus* belongs to the family *Veillonellaceae*, which is known for producing succinate, a potential microbe-derived metabolite associated with an increased risk of cardiovascular disease (51). The function of succinate may be in activating hypoxia-inducible factor HIF-1 α , a key transcription factor underlying chronic inflammation and dysfunction in obesity (52). Elevation of BMI has been described as an independent risk factor for adult and pediatric severe asthma (53,54). Additionally, a higher BMI increases the risk of asthma morbidity associated with respiratory tract infections (55). In our study, BMI positively correlated with the relative abundance of *Acidaminococcaceae* in the SA group (Figure 3D). We speculated that the *Acidaminococcaceae* family (*Firmutes* phylum) probably increased the incidence of asthma. The

characteristics of severe asthma in our study included the following: impairment of lung function (decreased MEF25% predicted), elevated eosinophil counts (in sputum), and elevated BMI. We considered that the intestinal flora may be closely related to the different levels of severity of asthma, given the correlation between clinical indicators and the relative abundance of intestinal flora.

In terms of the epidemiology and natural history of asthma, the outcomes and treatment regimens (TENOR) study and other studies have elucidated that total serum IgE levels are associated with asthma severity (56,57). At present, the anti-IgE antibody, omalizumab, has been recommended for use as an add-on therapy in severe asthma (9,58). Furthermore, the potential for indoor microbial dysbiosis to contribute to asthma severity is determined by serum IgE levels (59). In support of the hypothesis that the role of serum IgE levels in gut microbiome



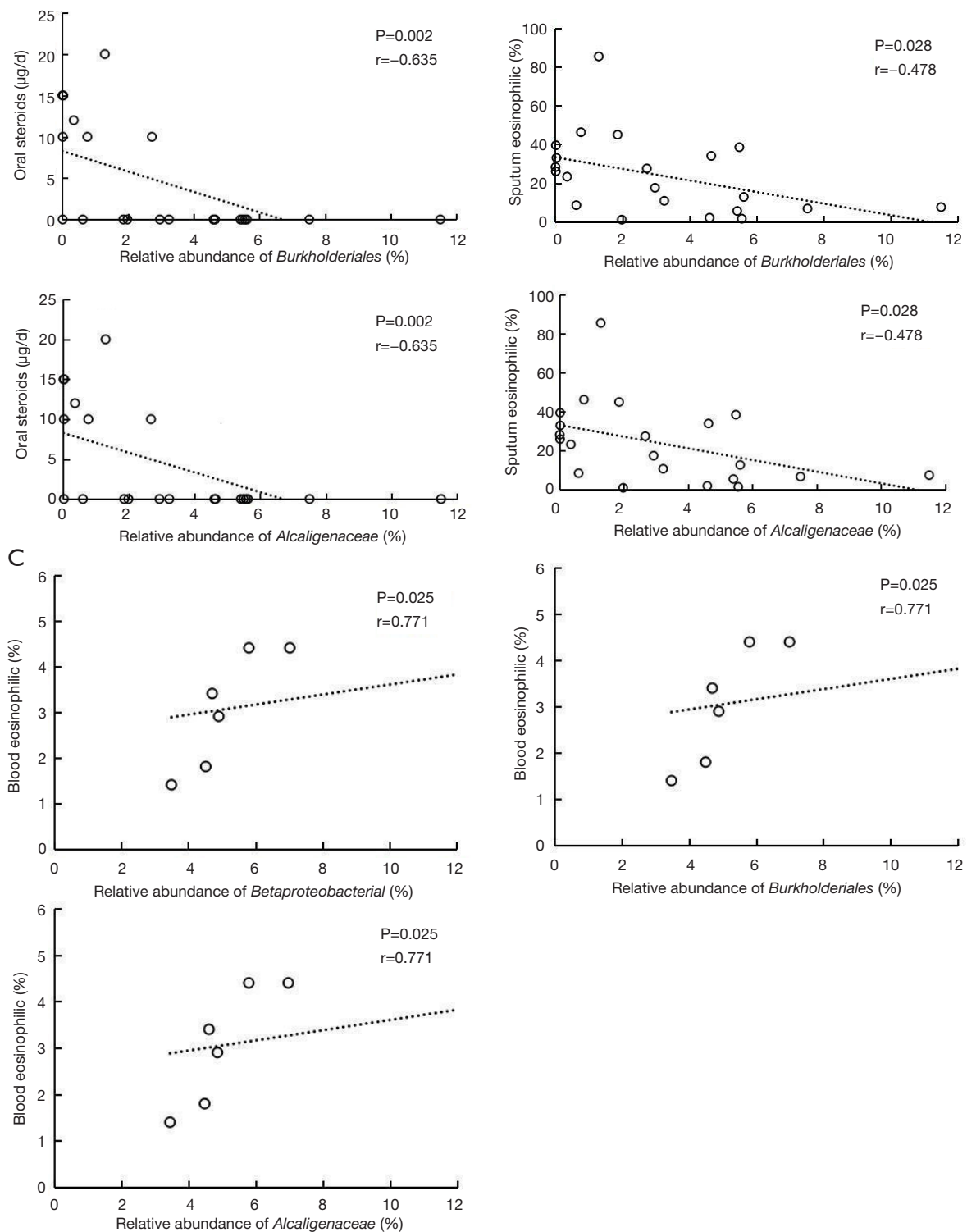


Figure 6 Scatter plots and correlations. (A) Scatter plot of the relative abundance of *Betaproteobacterial* class, *Burkholderiales* order, and *Alcaligenaceae* family shows positive correlations with nasal disease and total immunoglobulin E (IgE) in the high-IgE asthma (HEA) group. (B) Scatter plot of the relative abundance of *Betaproteobacterial* class, *Burkholderiales* order, and *Alcaligenaceae* family shows negative correlations with the dose of oral steroids and sputum eosinophils. (C) Scatter plot of the relative abundance of *Betaproteobacterial* class, *Burkholderiales* order, and *Alcaligenaceae* family shows positive correlations with blood eosinophils in the low-IgE asthma (LEA) group (Spearman's rank correlation coefficient, $P < 0.05$).

Table 5 Principal component analysis of the clinical features and relative abundance of microbiota

	Principal component (% of total variance)				
	1 (32.2%)	2 (16.0%)	3 (13.7%)	4 (11.5%)	5 (10.2%)
Diagnosis	0.407	0.188	0.701	-0.332	-0.064
Duration	0.143	0.862	-0.030	-0.094	-0.085
Nasal disease duration	0.020	0.825	0.247	0.031	0.072
Inhale corticosteroid	-0.848	-0.063	-0.140	0.212	-0.047
Sputum neutrophilia	0.833	0.096	0.128	0.124	0.109
Prevotellaceae	-0.084	-0.089	-0.930	-0.074	-0.131
Acidaminococcaceae	0.121	-0.017	0.080	0.010	0.982
Veillonellaceae	-0.045	-0.041	-0.045	0.961	0.008

dysbiosis of asthmatic subjects remains unknown. Our study divided all of the asthmatic patients into the HEA and LEA groups (Table 3). Some studies found that the range of serum total IgE in asthma fluctuated greatly (60-62). It was hard to obtain the accurate levels of serum IgE to define the asthma phenotypes. We selected 60 IU/mL as a cutoff value of serum total IgE on the basis of The Clinical Allergic Diseases (63). The features of the HEA group included poor management of asthma (high risk of exacerbation) and higher eosinophil levels (in sputum and blood). Compared with those in the LEA group, the relative abundances of *Betaproteobacteria* class, *Burkholderiales* order and *Alcaligenaceae* family were significantly low in the HEA group (Figure 5). We also found significant correlations between the bacterial abundances and clinical features in both the HEA and LEA groups (Figure 6).

Serum total immunoglobulin E is secreted by sensitized B-cells; the B-cells binds to an allergen and then to a receptor on mast cells or basophils, where they triggers the release of inflammatory mediators (64). It can be affected by metabolites of intestinal microflora which can maintain the immune regulatory T-cell survival and its function (64). On the one hand, it has been demonstrated that a modulation of HDM exposure (which elevated immunoglobulin E level) in case of microbial dysbiosis can change the gut microbial community (65). In addition, IgE is also produced locally in the gut; the particular subsets of airway microbiome dysbiosis-associated HDM-specific IgE response can interact with corresponding gut microbiome by changing the IgE levels in the gut through the gut-lung axis (65,66). On the other hand, the gut microbiome affects the levels of serum IgE depending on metabolites. Two

studies indicated that low intestinal *Proteobacteria* phylum during the first week and years of life was associated with IgE-associated eczema (67,68). Furthermore, there was a correlation between the decrease in the relative abundance of intestinal *Proteobacteria* phylum and serum IgE in patients with allergic diseases; the potential mechanism was that the molecular structure and metabolites of the intestinal flora were recognized by receptors TLR driving differentiation of Treg cells, which caused imbalance between Th1 and Th2 immune responses (47,48,67,68) and changed the serum IgE and eosinophils in sputum and blood (47). In contrast, Liu *et al.* suggested that higher levels of *Proteobacteria* phylum may lead to an increase in LPS content and increase the risk of inflammation and immunological abnormality, such as allergic rhinitis (69). In general, a potential relationship exists between the relative abundance of *Proteobacteria* phylum (including *Betaproteobacteria* class, *Burkholderiales* order, and *Alcaligenaceae* family) and serum total IgE from allergy disease; however, further studies are needed to fully understand the relations between gut microbiome dysbiosis and serum IgE levels in patients with asthma.

Finally, the OTUs of intestinal flora were extracted to conduct a principal component analysis with clinical indicators from subjects with asthma. The results showed that a higher dose of ICS was associated with more severe asthma. The implications of *Acidaminococcaceae* family, *Prevotellaceae* family, and *Veillonellaceae* family in specific phenotypes of severe asthma were also revealed by principal component analysis in our study (Table 5). This may help to take the intestinal flora as a biomarker to determine asthmatic phenotype.

This study had limitations. First, the current work was

based on a small sample size. Therefore, the effects of gut microbiome dysbiosis in severe asthma need to be further explored in studies with larger sample sizes and with extend duration of observations. Second, several confounding factors have also influenced gut microbiome dysbiosis, such as geographical region, diet, and indoor/outdoor pollution. Third, changes in food composition were associated with intestinal flora disorder, especially the ratio of fructose to glucose (14,15); thus, it would have been valuable to evaluate participants for fructose malabsorption via a hydrogen breath test. Finally, our study did not include paired sputum samples.

Overall, our present study revealed that altered gut microbiome may be associated with the severity of asthma. Furthermore, our findings may aid in further understanding the relationship between serum IgE levels and gut microbiome dysbiosis in asthmatic patients.

Conclusions

Gut microbiome plays an important role in the development of asthma. Altered gut microbiome composition is related to characteristics of asthma, including the severity of asthma and phenotypes defined by serum IgE levels.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all subjects, and the research was approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical University (approval No. 2016-68).

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