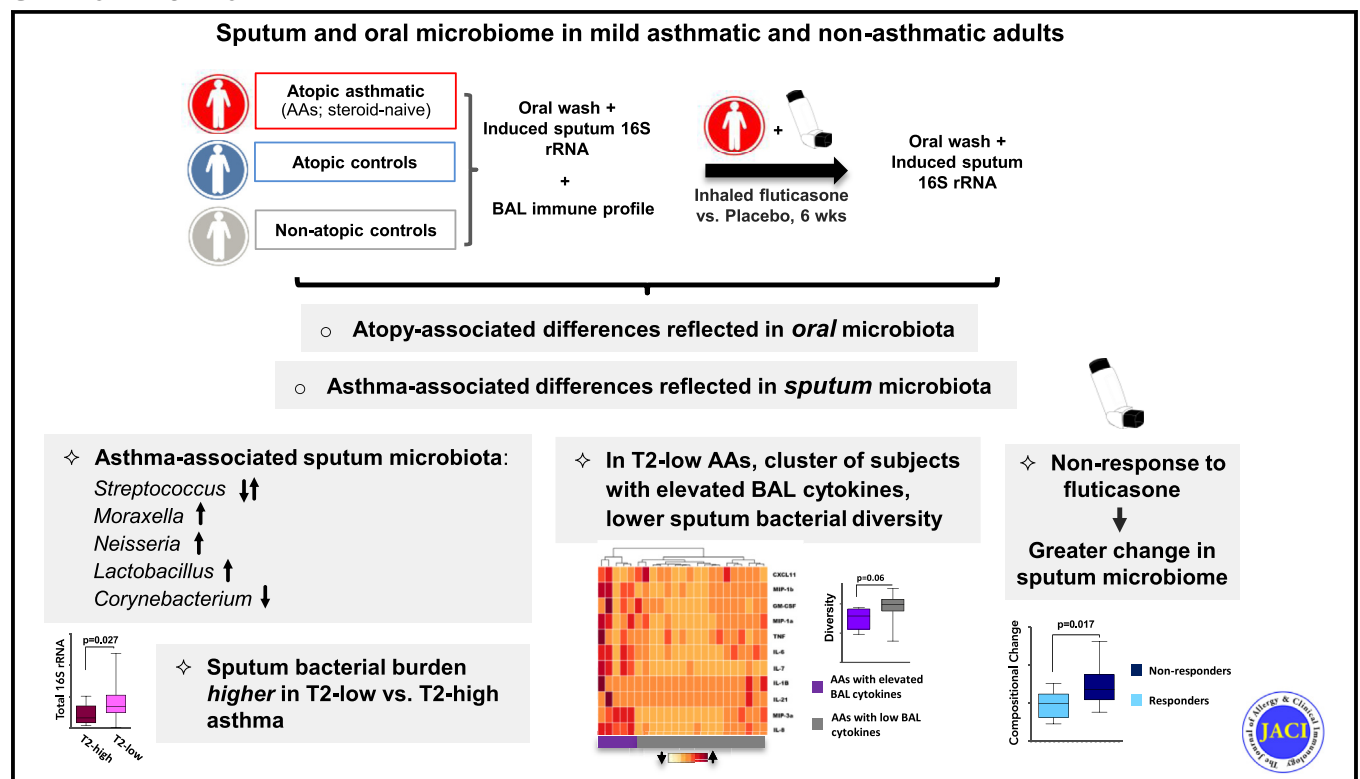


Distinct associations of sputum and oral microbiota with atopic, immunologic, and clinical features in mild asthma

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GRAPHICAL ABSTRACT



Background: Whether microbiome characteristics of induced sputum or oral samples demonstrate unique relationships to features of atopy or mild asthma in adults is unknown.

Objective: We sought to determine sputum and oral microbiota relationships to clinical or immunologic features in mild atopic asthma and the impact on the microbiota of inhaled

corticosteroid (ICS) treatment administered to ICS-naïve subjects with asthma.

Methods: Bacterial microbiota profiles were analyzed in induced sputum and oral wash samples from 32 subjects with mild atopic asthma before and after inhaled fluticasone treatment, 18 atopic subjects without asthma, and 16 nonatopic

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healthy subjects in a multicenter study (NCT01537133).

Associations with clinical and immunologic features were examined, including markers of atopy, type 2 inflammation, immune cell populations, and cytokines.

Results: Sputum bacterial burden inversely associated with bronchial expression of type 2 (T2)-related genes. Differences in specific sputum microbiota also associated with T2-low asthma phenotype, a subgroup of whom displayed elevations in lung inflammatory mediators and reduced sputum bacterial diversity. Differences in specific oral microbiota were more reflective of atopic status. After ICS treatment of patients with asthma, the compositional structure of sputum microbiota showed greater deviation from baseline in ICS nonresponders than in ICS responders.

Conclusions: Novel associations of sputum and oral microbiota to immunologic features were observed in this cohort of subjects with or without ICS-naïve mild asthma. These findings confirm and extend our previous report of reduced bronchial bacterial burden and compositional complexity in subjects with T2-high asthma, with additional identification of a T2-low subgroup with a distinct microbiota-immunologic relationship. (*J Allergy Clin Immunol* 2020;■■■:■■■-■■■.)

Key words: Microbiome, cytokines, sputum, oral, asthma, allergic, corticosteroids, type 2 inflammation

Perturbations in bronchial microbiota composition have been associated with several phenotypic features of asthma in adults, including airway hyperresponsiveness, airflow obstruction, response to steroids, and neutrophilic inflammation.¹⁻⁷ There is concurrent interest in phenotypic stratification of asthma based on underlying airway immune response patterns,⁸⁻¹⁰ and recent studies have described significant relationships between bronchial microbiota patterns and the level of type 2 (T2) airway inflammation present.^{3,11-13} We recently reported findings of reduced bacterial burden in bronchial epithelial brushings from

Abbreviations used

AA:	Atopic asthmatic
ANA:	Atopic nonasthmatic
BAL:	Bronchoalveolar lavage fluid
HC:	Healthy control
ICC:	Inflammatory cytokine cluster
ICS:	Inhaled corticosteroid
ICSR:	ICS responder
ICSNR:	ICS nonresponder
IS:	Induced sputum
OTU:	Operational taxonomic unit
OW:	Oral wash
TGM:	Three-gene mean

patients with mild asthma with a T2-high inflammation pattern,³ with few associations between specific bronchial bacteria and T2-related inflammatory markers.^{3,11} Although sampling the airways by bronchoscopy is desirable for many reasons, practical constraints limit its utility to investigate a broad range of asthma phenotypes in the larger population.

Studies of sputum have described associations between neutrophilic severe asthma and the relative abundance of organisms representing potential respiratory pathogens such as members of the *Moraxella* and *Haemophilus* genera.¹⁴⁻¹⁸ However, possible relationships between eosinophilic asthma and sputum microbiota remain obscure. Furthermore, treatment with inhaled corticosteroids (ICSs) has been shown to influence the composition of bronchial airway microbiota in patients with asthma,^{3,4,13,15} potentially affecting their clinical efficacy.^{3,4} It remains unclear whether ICS treatment similarly affects the oral and sputum microbiota. Finally, potential links between the oral microbiota and features of atopic asthma in adults have so far been overlooked, despite recent evidence of associations with risk for atopic disease, particularly asthma, in children.¹⁹

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We recently showed that although induced sputum (IS) microbiota composition shares features with that of oral wash (OW) samples, the former is nonetheless distinct and in subjects with mild asthma, more closely resembles that of bronchial brush samples.²⁰

Because IS and OW are more easily obtained, we here investigated whether relationships between specific clinical and immunologic features in mild atopic asthma and the microbiota in these sample types exist. We used samples collected in a multicenter study from which we previously reported such relationships with bronchial brush microbiota.³ We hypothesized that distinct characteristics of sputum and oral microbiota composition associate with phenotypic features in mild asthma, including immunologic measures related to atopy and/or asthma. We also hypothesized that ICS treatment of steroid-naïve subjects with mild asthma induces significantly different changes in the airway and oral microbiota between ICS-responsive and nonresponsive subjects.

METHODS

For additional details, please see this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Study population and sample collection

This study analyzed samples and data from a subset of adult subjects enrolled in a National Heart, Lung, and Blood Institute AsthmaNet microbiome study (NCT01537133)^{3,20} and included 32 atopic asthmatic (AA), 18 atopic nonasthmatic (ANA), and 16 nonatopic healthy control (HC) subjects, whose characteristics are summarized in [Table I](#) and [Table E1](#) in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org. Details of the study protocol have been previously described.³ At enrollment, all subjects were clinically stable for at least 3 months including no use of antibiotics; AA subjects had not used a controller medication in the previous 6 months. OW, IS, and bronchoalveolar lavage fluid (BAL) were collected at baseline, and in AA subjects also after treatment with inhaled fluticasone propionate or placebo twice daily for 6 weeks.³ Each subject provided informed consent approved by each clinical center's Institutional Review Board including use of their samples and data for subsequent analyses.

Bronchial epithelial "three-gene mean" score evaluation

Expression levels of 3 bronchial epithelial genes (*CLCA1*, *SERPIN2*, and *POSTN*) previously shown to be induced by IL-13²¹⁻²³ were measured to calculate the "three-gene mean" (TGM) score for each participant. T2-high asthma was defined as a TGM score of greater than or equal to 1.117 (2 SDs above the average TGM score in HC subjects), as previously described.³

BAL and blood cell flow cytometry, BAL cytokine multiplex testing

Blood samples for immunophenotyping were collected at bronchoscopy directly into Cyto-Chex BCT blood collection tubes (Streck, Inc, La Vista, Neb). BAL was collected and processed within 48 hours for immunophenotyping²⁴ and cytokine measurements, as detailed in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org. Among CD45⁺CD3⁺CD4⁺ T cells, cell populations enriched for functionally distinct T-cell subsets ([Table E1, A](#)) were identified as follows²⁴: regulatory T cells (CD25hiCD127lo), T_H2 (CCR6⁺CCR4⁺), T_H1 (CCR6⁺CCR4⁺), and T_H17 (CCR6⁺). BAL supernatants were concentrated and assayed using a MILLIPLEX MAP human high-sensitivity T-cell panel 21-plex immunology multiplex assay for detection of 21 targeted cytokines as previously described,²⁰ of which 11 had detectable levels ([Table E1, B](#)).

Nucleic acid extraction and 16S rRNA-based sequencing of OW and IS samples

Only IS samples with less than 80% squamous epithelial cell counts were included for bacterial community analysis. OW and IS samples were processed as previously described²⁰ for bacterial community profiling by sequencing of the V4 region of the 16S rRNA gene on an Illumina Miseq.

Sequence data processing and quality control

Sequence analysis was performed using the Quantitative Insights into Microbial Ecology pipeline,²⁵ as described previously.³ Operational taxonomic units (OTUs) were picked at 97% sequence identity using UCLUST²⁶ against the Greengenes²⁷ database. Reads that failed to hit the reference sequence collection were retained and clustered *de novo*. OTUs identified in negative processing controls (2% of total negative control reads) and those present at less than 0.001% of total counts were removed from the OTU table. Alpha-diversity indices and UniFrac distances were calculated using the OTU table multiply rarefied^{3,20} to 53,219 sequences per sample. All sequence data related to this study are available from the European Nucleotide Archive under accession numbers PRJEB15534 and PRJEB22676.

Statistical analysis

Statistical analyses were performed in Quantitative Insights into Microbial Ecology, R environment, or PRISM (San Diego, Calif) software. Nonparametric or parametric tests for between-group differences were selected on the basis of outcome of Shapiro-Wilk normality test. Results were corrected for multiple testing where appropriate using *q*-value or Benjamini-Hochberg method.²⁸ Principal-coordinate analyses were performed on unweighted and weighted UniFrac distance matrices and distance-based PERMANOVA^{29,30} used to identify factors that explained variation in bacterial community beta-diversity (between-samples). Negative binomial regression corrected for multiple testing (*q* < 0.10) was used to determine specific OTUs that differed in relative abundance between groups. Mantel test³¹ based on weighted UniFrac distance was used to compare community composition between paired samples in each treatment group.

RESULTS

Cohort characteristics

As reported previously,^{3,20} AA subjects in this cohort had mild well-controlled asthma and were more likely to report a history of atopic symptoms compared with ANA and HC subjects ([Table I](#)). AA subjects had significantly higher serum total IgE and blood eosinophil counts and were more likely to be multisensitized to aeroallergens than ANA subjects ([Table I](#) and [Table E1, A and B](#)), indicating an overall elevated atopic status in this group of subjects. AA subjects also displayed greater T2 airway inflammation based on their TGM score³ for IL-13-responsive epithelial genes in bronchial brush samples ([Table I](#)). Airway hyperresponsiveness associated with a higher TGM score, serum total IgE, and blood eosinophil percentage, but inversely with proportion of regulatory T cells in BAL fluid ([Table II](#)).

Sputum and oral microbiota characteristics associate with distinct clinical and inflammatory features in AA and ANA subjects

Neither bacterial burden nor alpha-diversity (ie, within-sample) measures of bacterial composition in sputum (IS) or OW differentiated the 3 groups of subjects (see [Table E2](#) in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org). However, there were distinct associations between alpha-diversity measures

TABLE I. Study cohort characteristics

Variable	AA subjects (n = 32)	ANA subjects (n = 18)	HC subjects (n = 16)	q value*	AA vs ANA or HC P value**
Age (y)	35 (27 to 42)	30 (25 to 44)	28 (25 to 48)	NS	
ACQ score (baseline)	0.6 (0.2 to 1.0)	—	—	—	
Age asthma diagnosed (y)	9 (5 to 23)	—	—	—	
% Males	41	50	50	NS†	
% Whites	56	56	69	NS‡	
Body mass index	26 (23 to 30)	25 (21 to 29)	26 (22 to 27)	NS	
FEV ₁ % predicted prealbuterol	89 (73 to 99)	99 (94 to 108)	100 (95 to 109)	0.017	.002 (AA vs ANA) .005 (AA vs HC)
FEV ₁ % predicted postalbuterol	101 (84 to 107)	104 (98 to 108)	102 (100 to 117)	NS	
Change in FEV ₁ %	7.5 (5.3 to 15.0)	3.0 (1.0 to 6.3)	4.5 (2.3 to 5.0)	0.001	<.001 (AA vs ANA) <.001 (AA vs HC)
PC ₂₀ (methacholine)	1.0 (0.2 to 2.4)	>32§	>32§	0.001	ND
Serum IgE (EU/mL)	169.5 (55.3 to 316.3)	45.0 (21.2 to 97.5)	14.5 (5.0 to 32.8)	0.001	.002 (AA vs ANA) <.001 (AA vs HC)
No. of positive sIgE	6 (2 to 9)	3 (2 to 4)	—	0.14†	
Atopic rhinitis (%)¶	57	22	0	0.004‡	ND
Eczema/atopic dermatitis (%)¶	41	22	0	0.059‡	ND
Blood neutrophils (%)	54 (49 to 61)	59 (51 to 65)	57 (52 to 65)	NS	
Blood eosinophils (absolute)	200 (100 to 400)	100 (82 to 175)	100 (75 to 200)	0.059	ND
Blood eosinophils (%)	4 (2 to 6)	2 (1 to 3)	2 (1 to 3)	0.017	.02 (AA vs ANA) .001 (AA vs HC)
Sputum neutrophils (%)	55 (33 to 65)	39 (22 to 50)	41 (29 to 73)	NS	
Sputum eosinophils (%)	0.4 (0.0 to 1.2)	0.0 (0.0 to 0.5)	0.0 (0.0 to 0.4)	NS	
TGM# score	0.4 (−0.1 to 1.2)	−0.4 (−0.7 to 0.1)	−0.9 (−1.2 to −0.1)	0.001	.002 (AA vs ANA) <.001 (AA vs HC)

All values are medians (IQR).

ACQ, Asthma Control Questionnaire; IQR, interquartile range; ND, not determined; sIgE, specific IgE.

*Statistical significance was determined using Kruskal-Wallis test with *q* values >0.150 assigned NS.

**Mann-Whitney test vs AAs.

†Statistical significance was determined using Mann-Whitney test with *q* values >0.150 assigned NS.

‡Statistical significance was determined using χ^2 test with *q* values >0.150 assigned NS.

§Methacholine challenge was stopped at 32 mg/dL and PC₂₀ for these subjects was censored.

||No. of positive sIgE (sIgE >0.35 kU/L) from a total of 12 aeroallergens tested by ImmunoCap assay.

¶Subjects with a positive history of allergic conditions specified (self-reported).

#TGM score—bronchial epithelial signature of type 2 inflammation as previously described, based on IL-13-responsive epithelial genes.

of either sample type and clinical and immunologic features (see Table E3 in this article's Online Repository at www.jacionline.org). Greater OW bacterial diversity correlated with better pulmonary function (FEV₁%) and with the proportion of BAL T_H1 (%CD45⁺) cells. In contrast, IS diversity correlated with neither of these measures, whereas IS richness correlated positively with the proportion of blood neutrophils. IS diversity (Shannon index) demonstrated a negative relationship with the proinflammatory cytokines IL-7 and IL-8 in BAL, although this was not statistically significant after adjustment for multiple comparisons (Table E3). These initial observations suggested differences in microbiota-immune interactions in the oral versus sputum compartments.

Beta-diversity measures, which capture between-sample differences in microbial composition, were used next to test differences in either IS or OW bacterial composition between the subject groups. Specifically, the phylogeny-based UniFrac distance was used including unweighted and weighted calculations that reflect, respectively, bacterial membership (ie, equally emphasizing low and high abundance taxa as present or absent) and their quantitative composition (whereby high overshadows low abundance taxa), thus revealing distinct attributes of the microbial assemblages. We observed that the bacterial membership of IS microbiota in AA subjects less resembled that of HC subjects than similarly compared IS microbiota in ANA subjects (difference in unweighted UniFrac distance, *P* = .03; see Fig E1 in this article's Online Repository at www.jacionline.org). This

suggested a bacterial dysbiosis gradient between the 2 atopic groups relative to nonatopic HC subjects. Interestingly, this feature was unapparent with oral microbiota, in which the bacterial membership of OW samples from either atopic group was equally dissimilar to HC subjects (Fig E1).

To test the hypothesis that compositional differences in OW or IS microbiota associate with specific BAL or blood immune markers of atopy and asthma, distance-based permutational analysis of variance (unweighted and weighted UniFrac) was performed. In both OW and IS samples, differences in bacterial community membership associated with postbronchodilator change in FEV₁%, Asthma Control Questionnaire scores, and BAL IL-7 levels (unweighted UniFrac, *P* < .05; see Table E4 in this article's Online Repository at www.jacionline.org). In IS samples only, bacterial community membership also associated with aeroallergen multisensitization and blood eosinophils (unweighted UniFrac, *P* < .05). In OW samples, bacterial community membership associated with study group, serum total IgE, as well as BAL IL-8 levels and proportion of BAL memory T cells and T_H17 T cells (unweighted UniFrac, *P* < .05; Table E4). When the abundances of bacterial taxa were considered (weighted UniFrac), IS bacterial community structure associated also with serum total IgE, proportion of BAL memory T cells, and BAL CXCL11 levels, suggesting that these associations are likely influenced by taxa present in higher abundance. These findings overall indicated differences in immune markers associated with distinct attributes of the

TABLE II. Significant associations between inflammatory markers and lung function measures of subjects with mild atopic asthma in this study

	PC ₂₀ (methacholine)			FEV ₁ % predicted prealbuterol		
	ρ^*	P value	BH P value†	ρ^*	P value	BH P value†
TGM score	−0.58	<.0001	<.001	−0.23	.066	.153
No. of positive specific IgE	−0.55	<.0001	<.001	−0.32	.009	.085
Serum IgE (EU/mL)	−0.49	<.0001	<.001	−0.27	.026	.140
Blood eosinophils (%)	−0.34	.007	.041	−0.33	.008	.085
Blood eosinophils (absolute)	−0.29	.022	.086	−0.21	.095	.197
Blood eosinophils (%CD45 ⁺)	−0.34	.008	.041	−0.31	.013	.086
Blood neutrophils (%CD45 ⁺)	.24	.064	.152	.39	.001	.034
BAL Treg cells (%CD3 ⁺ CD4 ⁺)	.28	.034	.104	.14	.269	.383
BAL T _H 1 (%CD45 ⁺)	.26	.042	.114	.23	.068	.153
Sputum eosinophils (%)	−0.27	.035	.104	−0.27	.031	.140

FDR, False-discovery rate; Treg, regulatory T.

*Analysis performed using Spearman correlation coefficient.

†FDR corrected for multiple comparisons using Benjamini-Hochberg method, with values ≤ 0.15 considered significant.

bacterial community configurations in sputum and OW samples. Ecologically, the oral cavity differs greatly from the respiratory tract despite some overlap in microbial constituents.^{20,32} Thus, the possibility exists for different interactions to occur between the microbiota and immune system within different mucosal compartments.

We then conducted taxon-level analyses comparing differences between the groups in the relative abundance of identified bacteria in IS or OW. Of IS bacterial OTUs most likely associated with asthma (ie, those present in similarly greater or lesser relative abundance compared with both control groups; Fig 1; see Tables E5–E7 in this article's Online Repository at www.jacionline.org), several belonged to genera previously reported to be asthma-associated, such as *Moraxella* and *Streptococcus*^{17,18,33–35} and *Lactobacillus*.¹ It is noted that a number of asthma-associated *Streptococcus* OTUs were depleted in asthmatic airways, highlighting likely species- or strain-level differences in members of this genus and asthma-related associations. In agreement with our earlier analysis of bronchial brushings,³ sputum enrichment of *Neisseria* appeared asthma-specific, and its relative abundance correlated with BAL IL-7 (see Fig E2 in this article's Online Repository at www.jacionline.org). Other IS taxa were found to be more likely associated with atopy (similarly enriched in AA and ANA subjects compared with HC subjects), specifically enrichment of *Granulicatella* and depletion of *Aggregatibacter* among others (Fig 1). Interestingly, specific taxa in IS, but not OW, correlated significantly with several immune markers in blood or BAL including IL-7 and IL-8 and proportion of BAL memory T cells (Fig E2). In agreement with these observations, no specific oral taxa were found to be asthma-specific (ie, present in similarly greater or lesser relative abundance compared with both control groups; Fig 1; see Tables E8–E10 in this article's Online Repository at www.jacionline.org). Instead, differences in specific oral taxa between the groups seemed more reflective of atopic status.

Sputum microbiota characteristics differ by T2 status among subjects with asthma

Bronchial epithelial TGM scores, reflective of IL-13–driven immune response, were higher in AA compared with non-AA

subjects (Fig 2, A). Compared with the T2-low AA subjects (n = 22), T2-high AA subjects (n = 9) had higher Asthma Control Questionnaire scores, serum IgE levels, and blood eosinophils and a lower proportion of circulating T_H17 cells (see Table E11 in this article's Online Repository at www.jacionline.org). T2-high AA subjects also demonstrated significantly lower bacterial burden in IS samples (Fig 2, B), confirming our previous finding from analysis of bronchial brushings.³ T2-high AA subjects also displayed a trend toward lower bacterial phylogenetic diversity in both IS and OW (Fig 2, C; see Fig E3, A, in this article's Online Repository at www.jacionline.org). Again, in comparison to T2-low AA subjects, sputum bacterial community structure in T2-high AA subjects appeared more dissimilar to that of the control group (Fig 2, D), a finding likely driven by the lower burden and diversity in T2-high AA subjects. In line with this, very few sputum bacterial taxa were enriched in T2-high compared with T2-low AA subjects, whereas conversely many more taxa were enriched in T2-low AA subjects, including members of *Haemophilus*, *Moraxella*, and *Neisseria* (Fig 2, E; see Tables E12–E16 in this article's Online Repository at www.jacionline.org; Fig E3, C). In contrast, oral microbiota composition in T2-low and T2-high subjects did not significantly differ in their similarity to the control groups without asthma (Fig E3, B), suggestive of no distinguishing relationships between OW bacterial composition and level of T2 inflammation.

T2-low patients with mild asthma with elevated lung proinflammatory cytokines exhibit lower sputum bacterial diversity

Given that subtypes of T2-low asthma are poorly understood but microbial associations implicated,^{16–18,36} we enquired whether distinct patterns of lower airway inflammation exist among the T2-low AA subjects in our cohort and, if so, whether these associate with bacterial community differences in IS or OW. Using unsupervised clustering we found that levels of immune cytokines in BAL differentiated T2-low AA subjects into 2 distinct clusters (Fig 3, A), with subjects in inflammatory cytokine cluster (ICC) 1 exhibiting elevated levels of IL-8, IL-6, IL-7, and MIP-3a ($q < 0.1$; see Table E17 in this article's Online Repository at www.jacionline.org).

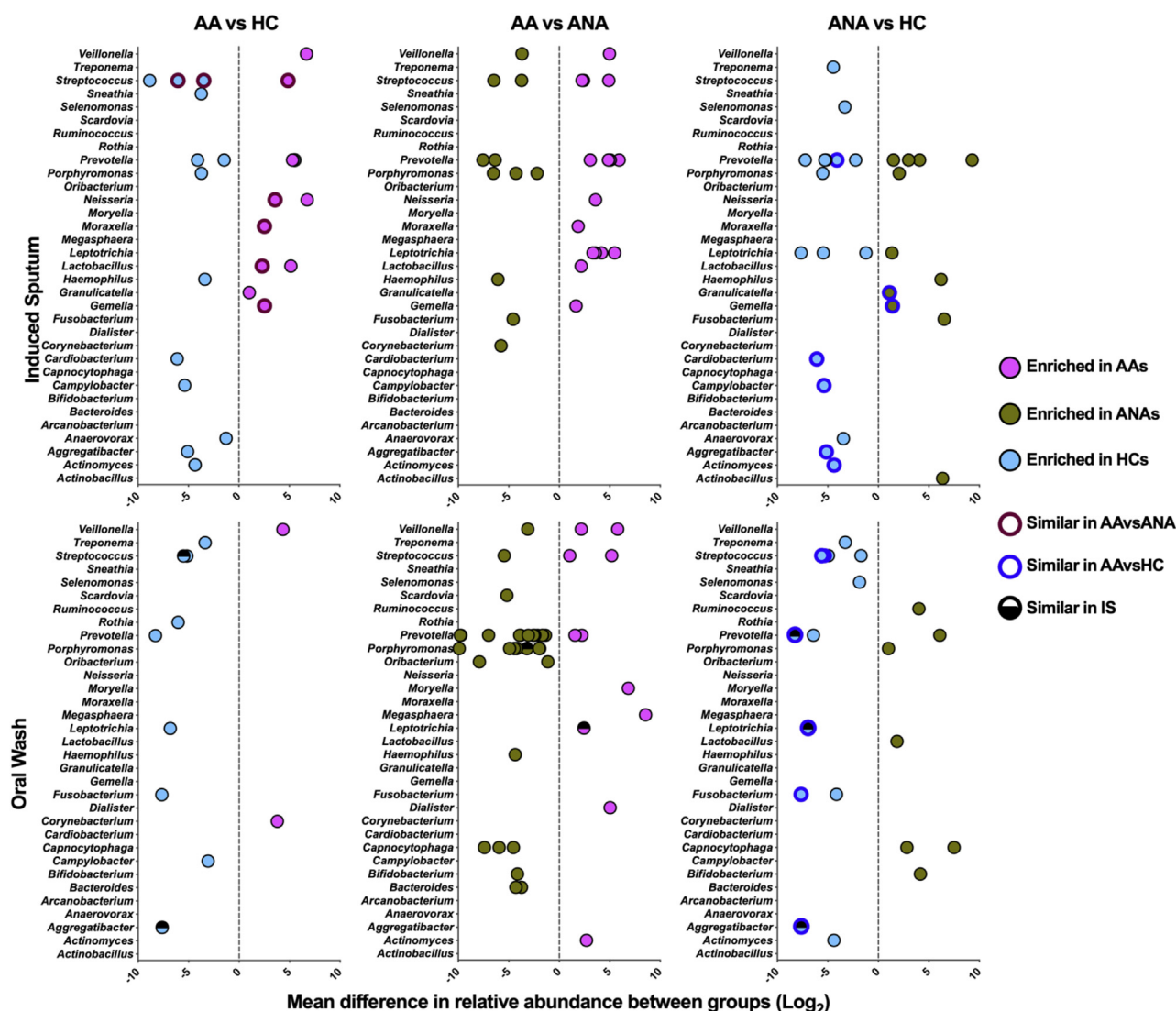


FIG 1. Mean difference in relative abundance (mean sequence reads) of specific bacterial taxa present in IS and OW in the 2-group comparisons shown (negative binomial regression model $q < 0.1$). Each dot represents a unique OTU within a given bacterial genus. Maroon colored circles highlight taxa most likely to be asthma-associated (ie, similarly enriched or depleted in AA vs ANA or HC subjects). Blue colored circles highlight taxa most likely to be atopy-associated (ie, similarly enriched or depleted in AA and ANA vs HC subjects).

Although both clusters harbored similar sputum bacterial burden and richness, ICC1 ($n = 5$) exhibited lower sputum bacterial diversity and evenness compared with ICC2 ($n = 18$; Fig 3, B), a pattern not observed with the oral microbiota (data not shown). These findings suggest possible loss of commensal bacteria and/or enrichment in specific microbiota members in ICC1 T2-low AA subjects. Analysis for such identified sputum depletion in *Treponema* in ICC1 compared with ICC2 T2-low AA subjects (Fig 3, C); oral enrichment in *Actinomyces* and *Atopobium* differentiated ICC2 from ICC1 T2-low AAs (see Fig E4 in this article's Online Repository at www.jacionline.org). Collectively, these observations suggest links between different lung inflammation patterns in subjects with T2-low mild asthma and the compositional structure of airway microbiota reflected here in sputum.

Shifts in sputum microbial community structure after inhaled corticosteroid treatment diverge between ICS responders and ICS nonresponders

Response to ICS treatment varies in asthma.^{3,4} We previously reported indications of a change in bronchial brush microbiota following fluticasone treatment that differed from placebo intervention among mild AA subjects.³ Here, on examining the same question in paired IS and OW samples available from a larger number of AA subjects, we found that sputum bacterial phylogenetic diversity trended toward a significant change after 6 weeks of fluticasone treatment ($n = 21$), with evidence of diversity loss compared with placebo controls ($n = 10$; see Fig E5 in this article's Online Repository at www.jacionline.org), a finding not observed in OW samples.

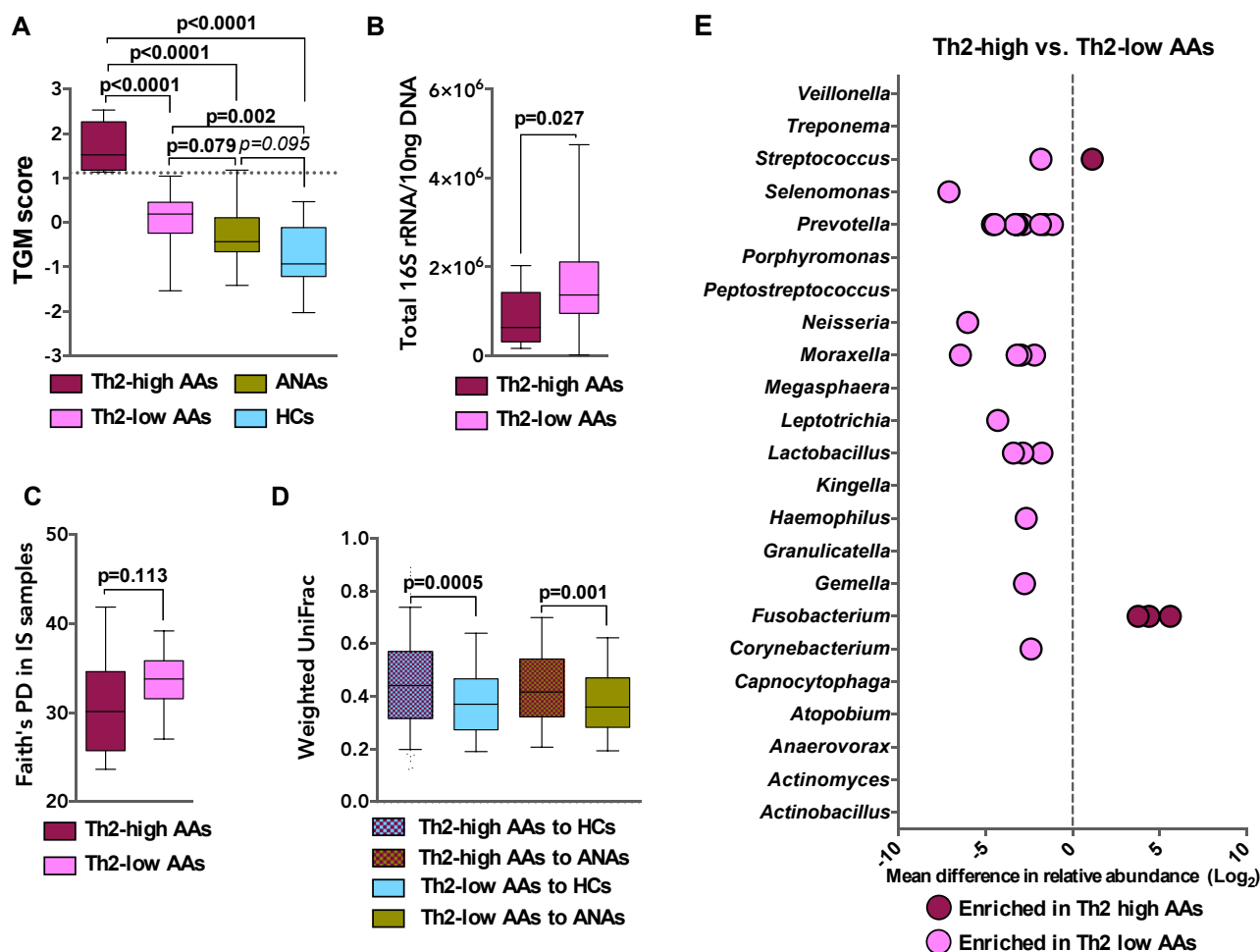


FIG 2. A, Greater expression of airway epithelial genes induced by T2 cytokines was observed in AA subjects, particularly in T2-high AA subjects ($n = 9$) with a TGM of 1.117 or greater (cutoff value indicated by a dashed line; Mann-Whitney test). B, Bacterial burden in IS of T2-high ($n = 9$) and T2-low ($n = 22$) AA subjects (Mann-Whitney test). C, Faith's phylogenetic diversity (PD) in IS samples of T2-high or T2-low AA subjects (Mann-Whitney test). D, Comparison of bacterial composition (weighted UniFrac) in IS of T2-high and T2-low AA subjects to that of controls without asthma (Bonferroni corrected t test; $P > .1$ not shown). E, Mean difference in relative abundance of specific bacterial taxa present in IS of T2-high vs T2-low AA subjects. Each dot represents a unique OTU within a given bacterial genus (negative binomial regression model $q < .1$).

As before,³ we classified the AA subjects by their change in methacholine PC₂₀ after ICS treatment: 12 AA subjects were ICS responders (ICSRs; ≥ 2 -fold increase in PC₂₀) and 8 nonresponders (ICSNRs). ICSNRs had lower FEV₁ and PC₂₀ values and higher circulating dendritic cells (see Table E18 in this article's Online Repository at www.jacionline.org). We hypothesized that bacterial compositional changes in ICSR and ICSNR subjects would diverge after fluticasone intervention. In the ICSR and placebo groups, the compositional structures of IS or OW microbiota at baseline and posttreatment were significantly similar at both time points (Mantel test; weighted UniFrac $P < .01$; see Table E19 in this article's Online Repository at www.jacionline.org). However, this was not the case in the ICSNR group for either IS or OW microbiota (Mantel test $P > .05$), indicative of a shift in bacterial community structure related to ICS intervention. Direct comparison of beta-diversity measures of microbiota composition between the time points

also demonstrated significantly greater deviation in sputum of ICSNR (Fig 4, A; $P < .05$). Fluticasone-associated changes in the relative abundance of specific bacterial taxa were identified, with some differences noted between ICSNR and ICSR (Fig 4, B; see Tables E20 and E21 and Fig E6 in this article's Online Repository at www.jacionline.org). Together these findings indicated a greater shift in the compositional structure of sputum microbiota after 6 weeks of intervention in ICSNRs.

DISCUSSION

The objective of this study, a secondary analysis of samples and data from participants in our previously reported trial,³ was to determine relationships between sputum and oral microbiota composition and clinical and immunologic features of atopy and asthma, the latter in subjects with mild ICS-naïve disease. Several observations were confirmatory; others are novel.

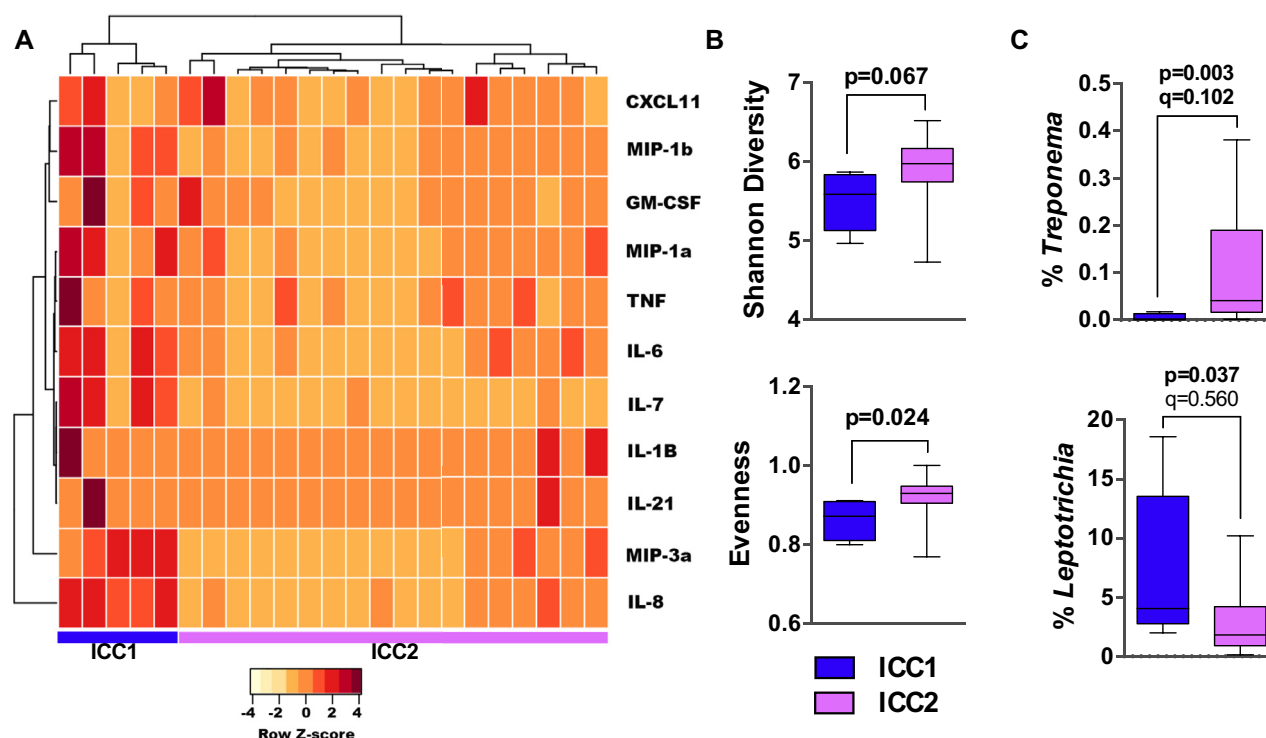


FIG 3. Altered bacterial microbiota composition of IS among "T2-low" AA subjects with elevated BAL inflammatory cytokines. **A**, BAL inflammatory cytokines differentiated T2-low AA subjects into 2 distinct clusters. Subjects in inflammatory cytokine cluster (ICC) 1 ($n = 5$) exhibited greater airway inflammation compared with ICC2 ($n = 18$). **B**, Bacterial diversity and evenness was lower in IS samples of T2-low ICC1 AA vs ICC2 AA subjects (Mann-Whitney test). **C**, Relative abundance of bacterial genera in IS, which differentiated ICC1 and ICC2 AA subjects (Mann-Whitney test, FDR-adjusted q values). FDR, False-discovery rate.

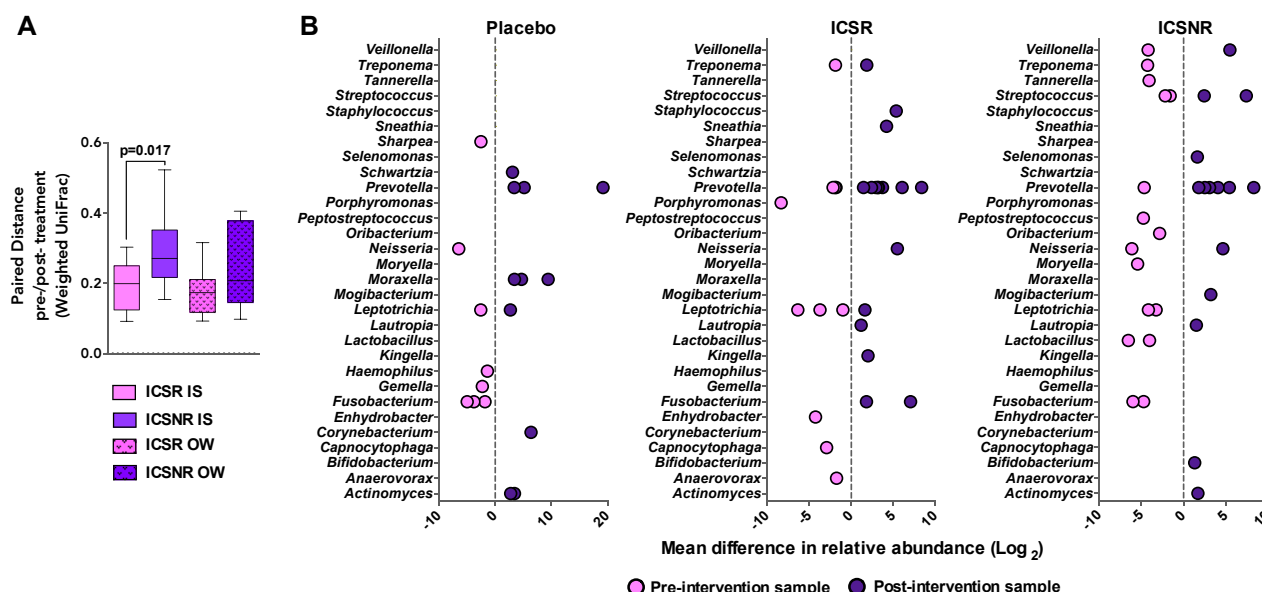


FIG 4. **A**, ICSNR subjects showed significantly greater change in paired weighted UniFrac distance in IS, but not OW, samples, compared with ICSRs (Mann-Whitney test). **B**, Mean difference in relative abundance of specific bacterial taxa present in IS of ICSR ($n = 12$) and ICSNR ($n = 8$) subjects, pre- and postintervention or in placebo-treated subjects with asthma ($n = 10$). Each dot represents a unique OTU within a given bacterial genus and whether it is enriched or depleted in the respective pre- or postintervention sample (negative binomial regression model $q < 0.1$).

We confirmed previously reported asthma-associated airway enrichment in *Neisseria* and *Moraxella*.^{3,5,33,34} We also confirmed a difference in airway bacterial burden (sputum) between T2-high and T2-low patients with asthma, which we previously observed in bronchial brushings.³ Moreover, we observed significant relationships between bacterial microbiota patterns in T2-low phenotype and clinical and immunologic measures. We identified a cluster of T2-low subjects with asthma, albeit small in number, characterized by significantly higher levels of several lung cytokines and reduced sputum bacterial diversity. Intriguingly, we also observed significant relationships between oral microbiota and specific clinical and immunologic features, most of which did not overlap with the relationships observed of sputum microbiota. We interpret the oral microbiota-associated findings as potentially more reflective of atopic status, and the sputum microbiota associations more reflective of asthma-associated immune responses in the lower respiratory tract.

Ecologically, the oral cavity is very different from the lower respiratory tract^{20,31} despite overlap in microbial constituents, and we previously reported from this cohort that the compositional structure of microbiota in our OW samples differed significantly from that of IS.¹⁹ Our OW sample consisted of collecting a mouth saline rinse (10 mL) plus tongue scrape material. This may have provided a broader picture of oral microbiota and contributed to the distinct associations with immune markers and atopic status, between OW and IS samples.

An important strength of this study was the collection of within-subject paired samples, enabling across-compartment comparisons of respiratory microbiota-immune marker relationships. We previously showed in this cohort that the compositional structure of microbiota in paired sputum and bronchial brush was more similar in subjects with asthma.²⁰ Hence, here we focused first on assessing sputum microbiota relationships to select immunologic features and the effect of ICS intervention. The same analysis was then performed with paired OW samples to explore their informativeness in revealing microbiota correlates with biological markers and outcomes of interest.

Differences in clinical and immunologic associations were seen between the 2 sample types, which we highlight as of interest for further study. For instance, IS alpha-diversity demonstrated a negative relationship, though not statistically significant after adjustment, with BAL IL-7 and IL-8, whereas OW alpha-diversity correlated with lower BAL IL-21, a higher proportion of BAL T_H1 cells, and higher FEV₁ %predicted. Both IL-7 and IL-21 are implicated in allergic immune cell responses,³⁷⁻⁴¹ including that of allergen-specific memory CD4 T cells and eosinophils. IL-7 can abrogate steroid inhibition of type 2 cytokine production by type 2 innate lymphoid cells.⁴¹ Furthermore, significant correlations between specific lung cytokines and specific microbiota members in IS, but not OW, were observed. These findings suggest that microbiota found in either oral or lower respiratory tract specimens could play a role in modulating immune responses and potentially reflect different aberrant mechanisms in atopy and asthma. Why or how atopy has an effect on the oral microbiome is not clear from our studies and will require further mechanistic investigations.

The design of our primary study³ was intended for examination of sputum and oral microbiome relationships to asthma clinical phenotypes. We focused on the categorization of subjects as either T2-high or T2-low based on a previously described IL-13-responsive epithelial gene expression signature.²¹⁻²³ Other biomarkers have been used to define the level of type 2 inflammation, but this approach was previously shown to perform well²¹ and also allowed for our earlier analysis of bronchial airway microbiota in the same specimen type.³ We noted that sputum bacterial burden in the T2-low group exhibited a broad range. Because this suggested possible bacterial heterogeneity among T2-low AA subjects, we further explored this leveraging our BAL cytokine data to examine microbial-immune relationships among T2-low AA subjects. This revealed 2 clusters defined by different lung cytokine patterns and associated differences in sputum bacterial diversity. Although the clinical significance of these findings is unclear from this exploratory analysis, the finding merits further study of these relationships in T2-low asthma.

Prospective administration of inhaled fluticasone led to ICS-associated changes in sputum bacterial community structure, which was more divergent in the ICSNR group. This suggests possible off-target effects of ICSs, particularly in this cohort of predominantly T2-low subjects with asthma. Although the treatment duration of 6 weeks was relatively short, our findings suggest the possibility that long-term ICS treatment in those unlikely to benefit could lead to persistent perturbation of the respiratory microbiome with potentially important clinical implications. Mechanisms for this are unclear. We previously found through *in silico* analysis a greater predictive capacity for xenobiotic biodegradation in bronchial microbiota of ICSNRs.³ A recent report also suggests that indirect mechanisms may play an important role, such as through ICS suppression of cathelicidin, an antimicrobial peptide, in the airways.⁴²

The important limitations of our multicenter study include the relatively small number of subjects in some of our subgroup analyses and the fact that determining cause-effect directionality of the relationships reported is inherently impossible in this clinical study. Sputum and oral samples both are also microbially complex and other nonbacterial microbiota were not assessed. Recently reported findings of differences in airway fungal composition associated with level of T2 inflammation support further investigation into nonbacterial members of the respiratory microbiome.¹³

Conclusions

In this cohort composed of AA, ANA, and HC subjects, we identified novel relationships between IS or oral microbiota characteristics and immunologic features measured in both BAL and peripheral blood samples. Findings from this study are hypothesis-generating and invite further study of the role that bacterial microbiota found in these respiratory tract compartments may play in shaping host immunity and clinical phenotypes of asthma, particularly those characterized by T2-low airway inflammation.

We acknowledge all AsthmaNet site investigators, study coordinators, and participants for their contributions to the parent study from which the data analyzed in this study derive.

Key messages

- Subjects with mild asthma with T2-high airway inflammation displayed significantly lower sputum bacterial burden compared with T2-low subjects, mirroring previous findings in protected brush specimens.
- Significant differences in sputum bacterial microbiota composition associated more with T2-low asthma phenotype, whereas differences in oral microbiota appeared more reflective of atopic status.
- A subgroup of T2-low subjects with mild asthma displayed elevations in several lung inflammatory mediators and an accompanying reduction in sputum bacterial diversity.
- After ICS treatment of subjects with mild asthma, the compositional structure of sputum microbiota demonstrated significantly greater deviation from baseline in ICSNRs than in ICSRs.

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SUPPLEMENTAL MATERIAL.**Methods***Study Population and Sample Collection.*

This study analyzed samples from a subset of subjects enrolled in the NHLBI AsthmaNet microbiome proof-of-concept study [NCT01537133; (1-3)] and included 32 atopic asthmatic (AA), 18 atopic non-asthmatic (ANA) and 16 non-atopic healthy control subjects (HC) whose characteristics are summarized in Table 1 and supplemental Table S1. Briefly, atopy was defined by serologic evidence (>0.35 kU/l) of sensitivity to ≥ 1 of 12 aeroallergens (specific IgE by ImmunoCap; Thermo-Scientific). Asthma was confirmed by measurement of airway responsiveness (methacholine $PC_{20} \leq 8$ mg/mL or FEV_1 improvement $\geq 12\%$ post-albuterol). At enrollment, asthmatics had been clinically stable for three months, without the use of a controller medication in the preceding six months. Exclusion criteria included a history of smoking, respiratory infection within six weeks or antibiotic use within three months of enrollment. All subjects underwent oral wash and sputum induction at a baseline visit, after which AA subjects were randomized in a 2:1 ratio to treatment with inhaled fluticasone propionate (250 mcg, GlaxoSmithKline) or placebo twice daily for six weeks and re-assessed post-treatment. Each subject provided informed consent approved by their center's IRB; an NHLBI-appointed Data Safety Monitoring Board (DSMB) oversaw the study conduct.

Samples processed for microbiota analysis were collected across nine clinical centers and included 66 baseline and 30 post-treatment pairs of oral wash (OW) and induced sputum (IS) samples. IS samples were collected and processed as previously described (1, 2) and only those with $<80\%$ squamous epithelial cell counts were included in this study. All samples intended for microbial analysis were stored in Saliva Protect reagent (Qiagen) to preserve nucleic acid at -80°C until processing.

“Three-gene mean” bronchial epithelial signature of type 2 inflammation.

Expression levels of three bronchial epithelial genes (*CLCA1*, *SERPINB2* and *POSTN*) previously shown to be induced by IL-13 (4, 5), were determined and the “three-gene mean” (TGM) score was calculated (6). A cut-off value of 1.117 (two standard deviations above the average TGM-score in healthy controls), additionally confirmed by unsupervised cluster and principal component analysis, was used (2) to classify asthmatic subjects as type 2 (T2)-“high” (n=9) or T2-“low” (n=22).

BAL and Peripheral Blood Cell Flow Cytometry

Blood samples (2 × 5 mL) for immunophenotyping were collected directly into Cyto-Chex BCT blood collection tubes (Streck). Bronchoalveolar lavage (BAL) was collected and processed for immunophenotyping and cytokine measurements. After removing aliquots for cell counting and microbiome analysis, remaining BAL was centrifuged at 300 g for 10 minutes. Cell pellets were resuspended and adjusted to 1 ml total volume in FACS buffer (PBS, 2mM EDTA, 0.1% w/v bovine serum albumin, 0.1% w/v sodium azide), then transferred into collection vials containing 1 mL Streck Cell Preservative (Streck). Within 48 hours, preserved BAL cells and peripheral blood mononuclear cells (PBMCs) were stained with fluorescently labeled antibodies as described previously (7). Samples were acquired with an LSR II flow cytometer (BD Biosciences) and analyzed with FlowJo software (TreeStar). Among CD45⁺CD3⁺CD4⁺ T cells, the following cell populations were identified as follows: regulatory T cells (CD25^{hi}CD127^{lo}), Th2 (CCR6-CCR4⁺), Th1 (CCR6-CCR4⁻) and Th17 (CCR6⁺).

BAL cytokine Multiplex Testing

BAL supernatants were concentrated and assayed using a MILLIPLEX MAP human high sensitivity T cell panel 21-plex immunology multiplex assay (Millipore) for detection of 21 targeted cytokines as previously described (1), of which 11 had detectable levels (Table S1b).

Nucleic acid extraction and 16S rRNA-based sequencing

Nucleic acids were extracted from IS and OW samples by methods identical to those used in the parent study (2). These involved using a modified bead-beating protocol and the AllPrep kit (QIAgen). The variable region 4 (V4) of the 16S rRNA gene was amplified using 515F/806R primer combination as previously described (8). Amplicons were purified using SPRI beads (Beckman Coulter), analyzed on Bioanalyzer (Agilent) and quantified using the Qubit HS dsDNA kit (Invitrogen). Blank control DNA extracts were also amplified, bead purified and sequenced for quality control. Samples with sufficient amplicon were pooled at 50 ng per sample. The barcoded, pooled library was quantified using the Qubit HS dsDNA kit (Invitrogen), denatured and 5pM was loaded onto the Illumina MiSeq cartridge (V3) in combination with a 15% (v/v) of denatured 12.5pM PhiX for sequencing.

Sequence data processing and quality control

Paired-end sequences were combined using FLASH version 1.2.7 (9). Sequence analysis was performed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline version 1.8.0

(10). Raw sequences were de-multiplexed by barcode and quality filtered by removing low quality sequences. Sequences with three or more consecutive bases with a Q score < 30 were truncated and discarded if the length was less than 75% of the original 250 bp read length. Sequences were aligned using PyNAST (11) and operational taxonomic units (OTUs) were picked at 97% sequence identity using UCLUST (12) against the Greengenes (13) database (version 13_5). Reads that failed to hit the reference sequence collection were retained and clustered de novo. PyNAST-aligned sequences were chimera checked using ChimeraSlayer and putative chimeras as well as OTUs present at 2% of total reads (total=1,553) identified in negative controls and those present at less than 0.001% of total counts were removed from the OTU table. A phylogenetic tree was built using FastTree (14) and used to compute Faith's Phylogenetic Diversity and UniFrac distances. Final working OTU table was normalized by multiply rarefying (1, 2) to 53,219 sequences per sample. All sequence data related to this study are available from the European Nucleotide Archive under accession numbers PRJEB15534 and PRJEB22676.

Statistical analysis:

All statistical analyses were performed as indicated in QIIME, R environment or using PRISM software. Statistical tests used were selected based on the outcome of Shapiro-Wilk normality test. Kruskal Wallis, Mann-Whitney, Chi-square or Fisher's exact tests were used to determine significant differences in comparisons between independent study groups. Results were corrected for multiple testing where appropriate using q-value or Benjamini-Hochberg method (15). Spearman correlation was used to test for an association between bacterial alpha diversity indices and immunological and clinical features. Principal coordinates analyses (PCoA) were performed on unweighted and weighted UniFrac distance matrices and non-parametric PERMANOVA (16) applied to identify factors that explained the variation in bacterial community beta-diversity. This particular algorithm permutes the input distance matrices to then calculate p-values for determination of significance (17), and as such further correction for multiple testing of variables typically is not applied. Negative binomial regression corrected for multiple testing ($q < 0.10$) was used to determine specific OTUs which differed in relative abundance (mean sequence reads) between groups. Mantel test (18) based on weighted UniFrac distance was used to compare community composition between paired samples in each treatment group.

Supplemental Data:**Table S1a:** Additional markers of systemic and lower airway inflammation assessed in the study by flow cytometry.

Variable	Allergic asthmatics AAs (n = 32)	Allergic non-asthmatics ANAs (n = 18)	Non-allergic non-asthmatics HCs (n = 16)	q-value
Blood neutrophils (%CD45⁺)	0.1 (0.1 - 0.8)	0.6 (0.2 - 1.9)	0.5 (0.3 - 0.7)	NS
Blood eosinophils (%CD45⁺)	1.7 (0.5 - 4.3)	1.2 (0.4 - 2.3)	0.3 (0.1 - 0.9)	0.030
Blood basophils (%CD45⁺)	0.9 (0.6 - 1.4)	1.3 (0.7 - 1.7)	0.9 (0.5 - 1.4)	NS
Blood pDC (%CD45⁺)	0.3 (0.1 - 0.4)	0.4 (0.3 - 0.4)	0.4 (0.2 - 0.6)	NS
Blood CD3⁺CD4⁻ T cells (%CD45⁺)	29.2 (24.7 - 32.4)	26.7 (19.8 - 35.1)	25.0 (21.1 - 31.9)	NS
Blood CD3⁺CD4⁺ T cells (%CD45⁺)	38.1 (34.2 - 43.0)	41.9 (29.9 - 51.5)	36.4 (31.0 - 52.8)	NS
Blood Tregs (%CD3⁺CD4⁺)	4.5 (3.1 - 5.2)	4.1 (3.1 - 4.6)	3.8 (3.1 - 4.6)	NS
Blood CD45RA⁺ naive T cells (%CD3⁺CD4⁺)	44.3 (30.0 - 50.4)	44.6 (33.1 - 54.4)	44.8 (41.4 - 51.4)	NS
Blood CD45RO⁺ memory T cells (%CD3⁺CD4⁺)	40.3 (34.3 - 53.3)	39.9 (32.6 - 45.9)	40.2 (31.2 - 41.8)	NS
Blood Th1 cells (%CD3⁺CD4⁺CD45RO⁺)	25.5 (19.9 - 32.2)	28.6 (20.8 - 33.4)	20.3 (15.4 - 26.5)	NS
Blood Th2 cells (%CD3⁺CD4⁺CD45RO⁺)	26.3 (21.6 - 31.6)	26.6 (20.9 - 31.6)	25.0 (21.5 - 28.4)	NS
Blood Th17 cells (%CD3⁺CD4⁺CD45RO⁺)	45.5 (38.9 - 52.1)	43.7 (32.8 - 53.0)	56.2 (35.2 - 63.4)	NS
BAL neutrophils (%CD45⁺)	0.1 (0.0 - 0.4)	0.1 (0.0 - 0.4)	0.0 (0.0 - 0.3)	NS
BAL eosinophils (%CD45⁺)	0.2 (0.0 - 0.8)	0.1 (0.0 - 0.5)	0.0 (0.0 - 0.3)	NS
BAL basophils (%CD45⁺)	0.02 (0.0 - 0.14)	0.01 (0.0 - 0.10)	0.01 (0.0 - 0.04)	NS
BAL pDC (%CD45⁺)	0.5 (0.2 - 0.9)	0.5 (0.2 - 0.8)	0.5 (0.1 - 1.0)	NS
BAL CD3⁺CD4⁻ T cells (%CD45⁺)	21.5 (15.4 - 35.3)	22.7 (13.1 - 45.7)	26.4 (14.6 - 44.1)	NS
BAL CD3⁺CD4⁺ T cells (%CD45⁺)	26.6 (9.4 - 43.7)	31.4 (16.6 - 62.1)	38.6 (26.0 - 53.3)	NS
BAL Tregs (%CD3⁺CD4⁺)	4.0 (2.0 - 6.5)	5.3 (3.9 - 8.0)	6.2 (3.5 - 8.8)	NS
BAL CD45RA⁺ naive T cells (%CD3⁺CD4⁺)	1.5 (0.4 - 5.5)	0.8 (0.5 - 1.7)	0.5 (0.0 - 3.6)	NS
BAL CD45RO⁺ memory T cells (%CD3⁺CD4⁺)	81.9 (67.2 - 87.1)	82.5 (73.6 - 88.2)	84.2 (76.4 - 89.8)	NS
BAL Th1 cells (%CD3⁺CD4⁺CD45RO⁺)	17.1 (14.0 - 20.6)	22.8 (17.0 - 32.1)	15.4 (10.8 - 18.7)	0.086
BAL Th2 cells (%CD3⁺CD4⁺CD45RO⁺)	20.1 (13.8 - 25.0)	27.9 (17.2 - 33.4)	18.0 (13.7 - 28.7)	NS
BAL Th17 cells (%CD3⁺CD4⁺CD45RO⁺)	59.6 (53.5 - 68.0)	49.2 (39.4 - 63.8)	66.2 (47.6 - 75.5)	NS

Table S1b. Measured BAL cytokines.

Variable	Allergic asthmatics AAs(n = 32)	Allergic non- asthmatic ANAs (n = 18)	Non-allergic non-asthmatic HCs (n = 16)	q-value
BAL GM-CSF (pg/mL)	339 (185 - 560)	381 (191 - 633)	223 (129 - 375)	NS
BAL IL6 (pg/mL)	78 (48 - 144)	106 (62 - 165)	82 (41 - 106)	NS
BAL IL7 (pg/mL)	0.3 (0.3 - 19.2)	0.3 (0.3 - 14.2)	0.3 (0.3 - 0.3)	NS
BAL IL8 (pg/mL)	1507 (843 - 2931)	1311 (863 - 2883)	1011 (556 - 1184)	NS
BAL CXCL11 (pg/mL)	371 (169 - 557)	485 (235 - 886)	390 (181 - 502)	NS
BAL MIP-1α (pg/mL)	85 (41 - 147)	106 (66 - 157)	73 (44 - 105)	NS
BAL MIP-1β (pg/mL)	257 (133 - 352)	319 (223 - 433)	226 (127 - 384)	NS
BAL MIP-3α (pg/mL)	436 (161 - 1488)	422 (314 - 884)	298 (183 - 1042)	NS
BAL TNF (pg/mL)	50 (16 - 86)	71 (44 - 103)	34 (23 - 56)	NS
BAL IL-1β (pg/mL)	0.04 (0.04 - 3.3)	0.04 (0.04 - 1.8)	0.04 (0.04 - 0.04)	NS
BAL IL21 (pg/mL)	0.04 (0.04 - 15.3)	4.1 (0.04 - 26.5)	0.04 (0.04 - 12.9)	NS

Table S2: Summary of bacterial alpha diversity measures assessed in sputum (IS) and oral wash (OW) microbiota.

Alpha diversity metric/ sample type	Allergic asthmatics AAs (n = 32)	Allergic non- asthmatics ANAs (n = 18)	Non-allergic non- asthmatics HCs (n = 16)	p- value ^{\$}
Observed species/IS	604 (529 - 630)*	599 (564 - 672) [#]	601 (528 - 645)*	NS
Observed species/OW	561 (510 - 618)	585 (523 - 618)	535 (493 - 616)	NS
Shannon index/IS	5.8 (5.5 - 6.1)*	6.0 (5.7 - 6.2)*	5.9 (5.4 - 6.2) [#]	NS
Shannon index/OW	5.4 (5.1 - 5.8)	5.6 (5.4 - 5.8)	5.4 (5.1 - 5.8)	NS
Faith PD/ IS	33 (30 - 35)*	33 (32 - 37)*	33 (30 - 36)*	NS
Faith PD/OW	32 (29 - 34)	32 (30 - 35)	31 (27 - 35)	NS
Pielou Index/IS	0.92 (0.87 - 0.95)*	0.94 (0.90 - 0.95)*	0.91 (0.97 - 0.96)*	NS
Pielou Index/OW	0.85 (0.82 - 0.90)	0.88 (0.86 - 0.91)	0.87 (0.81 - 0.90)	NS

^{\$}Significance was determined using Kruskal-Wallis test with p-values >0.05 assigned NS. Difference in the same alpha indices between paired IS and OW samples evaluated using Wilcoxon Matched-pairs signed rank test are indicated as *p<0.05 and [#]p<0.10.

Table S3: Significant associations between bacterial alpha-diversity indices of induced sputum (IS) and oral wash (OW), and clinical and inflammatory parameters of subjects in this study.

alpha diversity in IS	rho^s	p-value	BH p-value[#]	Clinical/Inflammatory variable
Observed species	0.323	0.009	0.132	Blood neutrophils (%CD45pos)
Shannon index	-0.264	0.032	0.287	BAL IL-7 (pg/mL)
Shannon index	-0.256	0.038	0.287	BAL IL-8 (pg/mL)
Pielou Index	-0.265	0.031	0.404	BAL IL-7 (pg/mL)
Faith index	0.286	0.021	0.314	Blood neutrophils (%CD45pos)
alpha diversity in OW				
Shannon index	0.382	0.002	0.025	BAL Th1 (%CD45pos)
Shannon index	0.281	0.022	0.130	FEV1 % pre-Ab
Shannon index	-0.261	0.034	0.165	BAL IL-21 (pg/mL)
Observed species	-0.271	0.028	0.488	BAL MIP-3a (pg/mL)
Faith index	-0.288	0.019	0.406	BAL MIP-3a (pg/mL)
Faith index	-0.252	0.041	0.406	BAL IL-8 (pg/mL)
Faith index	0.247	0.046	0.406	BMI
Pielou Index	0.413	0.001	0.009	BAL Th1 (%CD45pos)
Pielou Index	0.316	0.010	0.057	FEV1 % pre-Ab
Pielou Index	-0.259	0.036	0.149	BAL IL-21 (pg/mL)

*Analysis performed using Spearman correlation coefficient. [#]p-values were FDR corrected for multiple comparisons using Benjamini Hochberg method, with values ≤ 0.20 considered significant

Table S4: Factors that explain microbiota variance in sputum and oral wash samples. (distance-based PERMANOVA [16,17])

Variable	Induced sputum				Oral wash			
	Unweighted UniFrac		Weighted UniFrac		Unweighted UniFrac		Weighted UniFrac	
	R ²	p-value	R ²	p-value	R ²	p-value	R ²	p-value
Group	0.037	0.146	0.021	0.690	0.046	0.030	0.036	0.302
Total 16S rRNA burden	0.029	0.012	0.023	0.186	-	-	-	-
Race	0.106	0.014	0.096	0.204	0.088	0.145	0.078	0.458
Age (yrs)	0.021	0.092	0.060	0.005	0.013	0.688	0.028	0.135
TGM status (high/low)	0.035	0.244	0.028	0.504	0.044	0.040	0.049	0.156
Age asthma diagnosed (yrs)	0.028	0.619	0.074	0.065	0.036	0.282	0.113	0.018
Change in FEV₁%	0.026	0.027	0.009	0.707	0.027	0.034	0.022	0.213
ACQ Score	0.057	0.018	0.039	0.270	0.066	0.005	0.084	0.044
Serum IgE (EU/mL)	0.019	0.204	0.044	0.023	0.028	0.023	0.027	0.130
No. of positive sIgE	0.025	0.039	0.015	0.382	0.018	0.267	0.010	0.554
Blood eosinophils (absolute)	0.025	0.051	0.018	0.320	0.024	0.055	0.021	0.225
Blood neutrophils (%)	0.008	0.994	0.006	0.867	0.014	0.560	0.050	0.021
Blood eosinophils (%)	0.027	0.029	0.017	0.349	0.024	0.067	0.028	0.118
Blood eosinophils (%CD45pos)	0.025	0.044	0.024	0.192	0.021	0.122	0.018	0.314
Blood Tregs (%CD3⁺CD4⁺)	0.016	0.414	0.026	0.145	0.013	0.650	0.049	0.023
BAL Th1 (%CD45pos)	0.017	0.267	0.038	0.040	0.017	0.303	0.003	0.971
BAL basophils (%CD45pos)	0.013	0.675	0.014	0.432	0.025	0.050	0.025	0.172
BAL Th17 T cells (%CD3⁺CD4⁺CD45RO⁺)	0.015	0.521	0.013	0.526	0.026	0.033	0.020	0.271
BAL CD45RO⁺ memory T cells (%CD3⁺CD4⁺)	0.022	0.094	0.047	0.021	0.026	0.036	0.028	0.142
BAL CD45RO⁺ naive T cells (%CD3⁺CD4⁺)	0.021	0.121	0.042	0.041	0.026	0.034	0.018	0.313
BAL TNF (pg/mL)	0.012	0.796	0.025	0.181	0.021	0.113	0.036	0.052
BAL IL-21 (pg/mL)	0.019	0.188	0.031	0.093	0.024	0.052	0.036	0.067
BAL IL-7 (pg/mL)	0.034	0.006	0.020	0.265	0.026	0.044	0.011	0.598
BAL IL-8 (pg/mL)	0.014	0.484	0.029	0.090	0.027	0.030	0.024	0.190
BAL CXCL11 (pg/mL)	0.019	0.183	0.039	0.045	0.013	0.643	0.018	0.312

Tables S5: List of specific bacterial taxa that differ in relative abundance in sputum microbiota between atopic asthmatic (AA) and healthy control (HC) subjects.

OTU_IDs	AAs (mean seq reads)	HC (mean seq reads)	AA-HC (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
4440404	109.44	0.81	108.63	2.98E-67	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	
968873	108.72	0.81	107.91	0.008	Bacteroidetes	Flavobacteriia	Flavobacteriales	[Weeksellaceae]		
4318671	207.13	103.31	103.81	0.056	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar
525942	67.66	21.88	45.78	0.056	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
1004910	141.09	101.31	39.78	3.63E-28	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4428313	35.63	0.13	35.50	0.056	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
567427	37.44	8.06	29.38	0.073	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
4299448	13.44	1.44	12.00	0.056	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	
14920	6.06	0.25	5.81	2.69E-08	Firmicutes	Bacilli	Gemellales	Gemellaceae	Gemella	
OTU733	5.88	0.13	5.75	0.054	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	
288521	5.06	0.13	4.94	1.63E-05	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
42538	4.53	0.19	4.34	0.056	Tenericutes	Mollicutes	RF39			
OTU828	2.88	0.81	2.06	0.095	Firmicutes	Bacilli	Lactobacillales			
OTU231	2.66	0.63	2.03	0.051	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Granulicatella	
4468500	1.16	3.56	-2.41	8.81E-06	Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Anaerovorax	
1109709	3.88	6.63	-2.75	0.004	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
956702	1.50	11.88	-10.38	2.29E-35	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	influenzae
526131	2.72	13.88	-11.16	0.006	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
OTU1595	0.13	13.25	-13.13	0.047	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
29566	0.63	13.88	-13.25	5.52E-38	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Sneathia	
OTU1680	0.13	17.25	-17.13	0.047	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU391	0.44	17.81	-17.38	1.95E-39	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae		
4306356	1.78	22.00	-20.22	0.009	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	

OTU_IDs	AAs (mean seq reads)	HC (mean seq reads)	AA-HC (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
4334479	6.09	26.94	-20.84	0.021	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	
4412991	1.22	23.50	-22.28	0.095	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae		
4207350	15.34	49.13	-33.78	1.97E-88	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Aggregatibacter	
4297695	8.59	50.00	-41.41	0.080	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	
3678349	15.34	81.06	-65.72	0.029	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus
4442779	13.16	82.63	-69.47	0.051	Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Cardiobacteriaceae	Cardiobacterium	
4296424	75.28	207.56	-132.28	0.079	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	
4335450	1.03	391.44	-390.41	4.67E-251	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae		
4315974	183.91	642.19	-458.28	0.047	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	

Tables S6: List of specific bacterial taxa that differ in relative abundance in sputum microbiota between atopic asthmatic (AA) and non-asthmatic atopic control (ANA) subjects.

OTU_IDs	AAs (mean seq reads)	ANAs (mean seq reads)	AA- ANA (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
530206	100.88	39.94	60.93	0.008	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
4297119	62.34	17.50	44.84	0.034	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
521268	49.91	16.83	33.07	0.032	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
4440670	31.41	0.11	31.30	4.09E-13	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	
567427	37.44	7.39	30.05	0.023	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
4382476	38.81	9.61	29.20	0.034	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
70628	20.53	2.33	18.20	0.024	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
4299448	13.44	1.33	12.10	0.023	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	
1033013	12.06	0.17	11.90	3.18E-11	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
923032	10.59	0.44	10.15	0.074	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
4426165	22.88	14.44	8.43	0.000	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU900	10.31	5.00	5.31	0.014	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
3581175	5.31	0.11	5.20	0.034	Firmicutes	Clostridia	Clostridiales	Veillonellaceae		
OTU66	4.91	0.11	4.80	1.09E-05	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
288521	5.06	0.56	4.51	2.12E-09	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
OTU733	5.88	2.22	3.65	2.80E-06	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	
14920	6.06	2.89	3.17	0.0002	Firmicutes	Bacilli	Gemellales	Gemellaceae	Gemella	
OTU95891	1.09	5.67	-4.57	0.0018	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
4310208	4.19	17.00	-12.81	0.0241	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	parvula
526131	2.72	16.00	-13.28	0.0010	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
4412630	4.03	23.44	-19.41	0.0004	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	

OTU_IDs	AAs (mean seq reads)	ANAs (mean seq reads)	AA- ANA (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
2438396	36.84	60.50	-23.66	1.39E-29	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
4337383	1.03	37.17	-36.14	0.006	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae		
964220	17.69	72.00	-54.31	0.010	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	durum
4469627	7.25	74.44	-67.19	3.05E-232	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	
114813	2.47	83.94	-81.48	2.60E-202	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
3678349	15.34	104.11	-88.77	0.009	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus
1029036	7.94	99.22	-91.28	0.061	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
4428060	48.84	236.56	187.71	0.006	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica

Tables S7: List of specific bacterial taxa that differ in relative abundance in sputum microbiota between atopic non-asthmatic (ANA) and healthy control (HC) subjects.

OTU_IDs	ANAs (mean seq reads)	HC (mean seq reads)	ANA- HC (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
4310398	847.7	223.9	623.8	0.022	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	nanceiensis
828676	103.1	11.4	91.7	1.57E-174	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
4318122	278.7	196.7	82.0	5.15E-51	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Actinobacillus	porcinus
4469627	74.4	0.9	73.6	2.47E-59	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	
OTU1635	22.7	5.8	16.9	0.023	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	nanceiensis
OTU1465	13.8	5.9	8.0	8.77E-11	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	intermedia
OTU95891	5.7	1.5	4.2	0.086	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
OTU501	4.2	0.8	3.4	0.037	Firmicutes	Clostridia	Clostridiales			
OTU64879	3.4	0.6	2.8	0.044	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	nanceiensis
14920	2.9	0.3	2.6	9.68E-05	Firmicutes	Bacilli	Gemellales	Gemellaceae	Gemella	
4335447	3.1	0.6	2.5	8.35E-05	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
OTU231	2.8	0.6	2.2	0.037	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Granulicatella	
OTU1062	0.2	2.6	-2.3	0.038	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
4477971	4.2	6.8	-2.6	0.038	Firmicutes	Clostridia	Clostridiales			
760967	1.9	6.7	-4.7	1.12E-08	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU1739	0.1	5.8	-5.7	0.046	Firmicutes	Clostridia	Clostridiales	Veillonellaceae		
253550	3.8	10.9	-7.2	6.64E-12	Bacteroidetes	Bacteroidia	Bacteroidales			
4459414	0.5	10.5	-10.0	0.004	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Selenomonas	noxia
790466	2.9	13.9	-11.0	1.73E-22	Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Anaerovorax	
3581175	0.1	14.1	-14.0	0.011	Firmicutes	Clostridia	Clostridiales	Veillonellaceae		
OTU1680	0.8	17.3	-16.4	2.61E-28	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU391	0.8	17.8	-17.0	2.56E-28	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae		
OTU1053	0.7	17.9	-17.3	4.42E-27	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica

OTU_IDs	ANAs (mean seq reads)	HC (mean seq reads)	ANA- HC (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
4334479	6.0	26.9	-20.9	0.095	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	
669126	0.7	22.8	-22.0	0.092	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	
4207350	13.6	49.1	-35.6	7.44E-67	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Aggregatibacter	
70671	17.9	54.7	-36.7	1.71E-63	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4382476	9.6	48.8	-39.2	0.036	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4297695	8.3	50.0	-41.7	0.080	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	
923032	0.4	44.1	-43.7	0.006	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
2613485	49.3	94.9	-45.5	5.71E-52	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
4442779	14.3	82.6	-68.3	0.0527987	Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Cardiobacteriaceae	Cardiobacterium	
245523	2.4	122.2	-119.7	6.80E-143	Bacteroidetes	Bacteroidia	Bacteroidales			
114813	83.9	235.1	-151.1	8.83E-248	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4297119	17.5	217.9	-200.4	4.98E-05	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
4335450	0.1	391.4	-391.4	5.62E-17	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae		

Tables S8: List of specific bacterial taxa that differ in relative abundance in oral microbiota between atopic asthmatic (AA) and healthy control (HC) subjects.

OTU_IDs	AAs (mean seq reads)	HC (mean seq reads)	AA-HC (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
4440670	20.34	0.06	20.28	1.94E-06	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	
4404731	14.34	0.56	13.78	2.25E-19	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	
4362073	0.34	8.75	-8.41	0.047	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	
669126	0.09	10.38	-10.28	0.009	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	
1059655	8.16	44.31	-36.16	0.023	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
3678349	5.88	51.00	-45.13	0.067	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus
4317340	21.25	86.94	-65.69	0.076	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Rothia	aeria
4297119	31.28	144.19	-112.91	0.093	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
4207350	7.19	203.56	-196.38	0.000	Proteobacteria	Gamma proteobacteria	Pasteurellales	Pasteurellaceae	Aggregatibacter	
2438396	0.69	201.88	-201.19	0.009	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
114813	2.34	313.75	-311.41	0.076	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	

Tables S9: List of specific bacterial taxa that differ in relative abundance in oral microbiota between atopic non-asthmatic (ANA) and healthy control (HC) subjects.

OTU_IDs	ANAs (mean seq reads)	HC (mean seq reads)	ANA- HC (mean seq reads)	q- value	Phylum	Class	Order	Family	Genus	Species
271159	2014.22	1064.00	950.22	0.094	Firmicutes	Bacilli	Lactobacillales			
4392350	199.39	18.94	180.45	0.006	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	
4431355	98.39	9.81	88.58	0.013	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae		
239506	158.67	90.75	67.92	1.35E-64	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4347159	18.06	0.19	17.87	5.75E-13	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	adolescentis
2724175	16.28	0.06	16.22	3.55E-06	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]	gnavus
250288	14.11	3.31	10.80	1.67E-19	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae		
4326452	10.50	1.63	8.88	0.074	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar
4451646	7.56	0.44	7.12	0.064	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	
4428313	3.72	0.13	3.60	0.000	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
OTU149750	2.50	0.50	2.00	0.051	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
936798	0.33	3.63	-3.29	3.55E-06	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus
4432347	0.11	3.75	-3.64	0.016	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Selenomonas	
4459021	1.56	11.25	-9.69	0.094	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Tannerella	
4448731	2.61	20.31	-17.70	0.011	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
4317477	0.94	22.00	-21.06	0.008	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	
4344207	6.17	37.06	-30.90	0.074	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus
1059655	4.61	44.31	-39.70	0.002	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
3678349	2.17	51.00	-48.83	0.001	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus
245523	0.06	83.25	-83.19	0.051	Bacteroidetes	Bacteroidia	Bacteroidales			
4428060	18.44	104.56	-86.12	0.026	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
4297119	19.00	144.19	-125.19	0.005	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	

OTU_IDs	ANAs (mean seq reads)	HC (mean seq reads)	ANA- HC (mean seq reads)	q- value	Phylum	Class	Order	Family	Genus	Species
2438396	0.44	201.88	-201.43	2.07E-64	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
4207350	1.61	203.56	-201.95	2.16E-145	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Aggregatibacter	
114813	0.22	313.75	-313.53	0.008	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	

Tables S10: List of specific bacterial taxa that differ in relative abundance in oral microbiota between atopic asthmatic (AA) and non-asthmatic atopic control (ANA) subjects.

OTU_IDs	AAs (mean seq reads)	ANAs (mean seq reads)	AA- ANA (mean seq reads)	q- value	Phylum	Class	Order	Family	Genus	Species
511378	532.59	156.83	375.76	0.042	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Megasphaera	
714766	150.69	35.78	114.91	0.063	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Moryella	
757622	75.47	20.44	55.02	0.073	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar
930592	60.91	24.28	36.63	0.063	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
403701	43.03	10.28	32.75	0.075	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Dialister	
4317477	7.41	0.94	6.46	0.063	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	
923032	5.69	0.22	5.47	0.073	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
1541939	6.13	1.39	4.74	0.096	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4445508	4.59	0.06	4.54	0.002	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	
OTU1401	7.69	3.22	4.47	0.093	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Arcanobacterium	
OTU923	4.13	1.17	2.96	0.035	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
586458	3.06	1.00	2.06	0.045	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
OTU1210	0.31	2.50	-2.19	0.043	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	
OTU64879	1.41	4.00	-2.59	0.096	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	nanceiensis
OTU43086	1.41	4.56	-3.15	0.075	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	nanceiensis
OTU997	0.66	3.83	-3.18	0.019	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
530164	1.41	5.22	-3.82	0.052	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
OTU438	2.06	5.94	-3.88	0.073	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
OTU898	1.00	5.00	-4.00	0.073	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
OTU442	1.72	6.11	-4.39	0.075	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]	
4406303	1.44	6.83	-5.40	0.029	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU921	0.81	6.72	-5.91	0.010	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	nanceiensis
OTU1489	3.22	11.56	-8.34	0.075	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	

OTU_IDs	AAs (mean seq reads)	ANAs (mean seq reads)	AA- ANA (mean seq reads)	q- value	Phylum	Class	Order	Family	Genus	Species
4326452	1.81	10.50	-8.69	0.019	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar
4412630	2.53	11.67	-9.14	0.009	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
3600504	0.19	13.56	-13.37	0.063	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	
4295238	3.00	17.39	-14.39	0.035	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]	
OTU1398	4.09	19.17	-15.07	0.042	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]	
4347159	0.38	18.06	-17.68	5.92E-37	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	adolescentis
OTU517	6.00	24.72	-18.72	0.063	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
4343627	0.09	19.72	-19.63	0.031	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	fragilis
2654263	12.72	33.22	-20.50	0.073	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	parainfluenzae
4409545	11.28	33.39	-22.11	0.056	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
1010329	0.88	24.06	-23.18	0.079	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	
OTU328	15.13	45.44	-30.32	0.043	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
4411875	3.06	39.50	-36.44	0.063	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Scardovia	
4297222	3.53	47.11	-43.58	0.052	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
4330572	24.81	86.44	-61.63	0.063	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	
4431355	17.47	98.39	-80.92	0.029	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae		
548730	23.75	151.39	-127.64	0.063	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4392350	29.63	199.39	-169.76	0.045	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	
749837	230.97	469.89	-238.92	0.015	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Oribacterium	
799211	220.03	1075.83	-855.80	0.073	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]	
4310398	452.28	1545.00	1092.72	0.041	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	nanceiensis
4321559	891.22	3046.72	2155.50	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	

Table S11. Clinical and inflammatory characteristics of T2- high and T2-low asthmatics.

Variable	T2-high AAs (n=9)	T2-low AAs (n=22)	q-value
Age (yrs)	27 (24 - 33)	38 (28 - 45)	NS
ACQ Score (Baseline)*	1.0 (0.8 - 1.3)	0.4 (0.1 - 0.7)	0.145
Age asthma diagnosed (yrs)	9 (5 - 24)	9 (5 - 22)	NS
Asthma duration (yrs)	18 (7 - 22)	24 (18 - 33)	NS
% Male	44%	41%	NS
% White	56%	59%	NS
BMI	26 (23 - 30)	26 (23 - 29)	NS
FEV1 % predicted pre-Ab [†]	88 (65 - 101)	88 (76 - 101)	NS
FEV1 % predicted post-Ab [†]	101 (90 - 109)	99 (84 - 106)	NS
Change in FEV%	15 (6 - 25)	7 (5 - 12)	NS
PC ₂₀ (methacholine)	0.8 (0.2 - 3.5)	1.2 (0.3 - 2.8)	NS
Serum IgE (EU/mL)	328 (166 - 641)	107 (43 - 214)	0.145
No. of positive sIgE [‡]	7 (2 - 11)	6 (2 - 9)	NS
Atopic rhinitis (%) [§]	56%	60%	NS
Eczema/Atopic dermatitis (%) [§]	44%	36%	NS
Blood neutrophils (%)	55 (44 - 62)	54 (48 - 61)	NS
Blood eosinophils (absolute)	400 (280 - 600)	175 (100 - 293)	0.145
Blood eosinophils (%)	7 (4 - 11)	3 (2 - 5)	0.093
Sputum neutrophils (%)	54 (34 - 65)	56 (33 - 66)	NS
Sputum eosinophils (%)	1.3 (0.4 - 3.8)	0.3 (0.0 - 0.9)	NS
TGM [§] score	1.5 (1.2 - 2.3)	0.2 (-0.2 - 0.5)	0.006
Blood Th1 (%CD45 ⁺)	4.4 (2.7 - 5.8)	4.2 (2.2 - 5.2)	NS
Blood Th2 (%CD45 ⁺)	4.4 (2.5 - 6.3)	4.2 (2.7 - 5.4)	NS
Blood Th17 (%CD45 ⁺)	4.4 (2.5 - 6.3)	7.9 (6.1 - 10.5)	0.103
Blood neutrophils (%CD45 ⁺)	0.1 (0.1 - 0.3)	0.2 (0.1 - 1.0)	NS
Blood eosinophils (%CD45 ⁺)	6.6 (1.1 - 9.3)	1.3 (0.5 - 2.8)	NS
Blood basophils (%CD45 ⁺)	0.9 (0.5 - 1.5)	0.9 (0.6 - 1.3)	NS
Blood pDC (%CD45 ⁺)	0.3 (0.1 - 0.4)	0.3 (0.1 - 0.5)	NS
Blood Th1 cells (%CD3 ⁺ CD4 ⁺ CD45RO ⁺)	30.5 (21.9 - 34.9)	23.5 (19.1 - 31.6)	NS
Blood Th2 cells (%CD3 ⁺ CD4 ⁺ CD45RO ⁺)	30.0 (22.1 - 34.3)	26.0 (21.7 - 31.5)	NS
Blood Th17 cells (%CD3 ⁺ CD4 ⁺ CD45RO ⁺)	41.9 (33.1 - 48.7)	46.9 (41.4 - 59.5)	NS
Blood CD45RO ⁺ memory T cells (%CD3 ⁺ CD4 ⁺)	38.0 (29.2 - 51.6)	41.1 (34.8 - 55.9)	NS
Blood CD45RA ⁺ naive T cells (%CD3 ⁺ CD4 ⁺)	46.8 (37.4 - 53.6)	42.5 (23.5 - 50.1)	NS

Variable	T2-high AAs (n=9)	T2-low AAs (n=22)	q-value
Blood Tregs (%CD3 ⁺ CD4 ⁺)	4.9 (2.8 - 6.1)	4.3 (3.0 - 4.9)	NS
Blood CD3 ⁺ CD4 ⁺ T cells (%CD45 ⁺)	42.6 (38.2 - 44.0)	36.1 (34.0 - 41.4)	NS
Blood CD3 ⁺ CD4 ⁻ T cells (%CD45 ⁺)	29.0 (24.8 - 33.9)	29.4 (23.4 - 32.7)	NS
BAL Th1 (%CD45 ⁺)	1.6 (0.6 - 3.3)	3.8 (1.7 - 7.8)	NS
BAL Th2 (%CD45 ⁺)	2.0 (0.6 - 3.8)	4.1 (1.8 - 7.6)	NS
BAL Th17 (%CD45 ⁺)	5.6 (2.0 - 18.0)	13.0 (5.7 - 23.2)	NS
BAL neutrophils (%CD45 ⁺)	0.4 (0.2 - 1.1)	0.1 (0.0 - 0.3)	NS
BAL eosinophils (%CD45 ⁺)	0.7 (0.2 - 1.0)	0.1 (0.0 - 0.6)	NS
BAL basophils (%CD45 ⁺)	0.0 (0.0 - 0.2)	0.0 (0.0 - 0.1)	NS
BAL pDC (%CD45 ⁺)	0.5 (0.0 - 1.3)	0.4 (0.2 - 0.9)	NS
BAL Th1 cells (%CD3 ⁺ CD4 ⁺ CD45RO ⁺)	17.2 (14.6 - 219.6)	16.7 (13.1 - 20.7)	NS
BAL Th2 cells (%CD3 ⁺ CD4 ⁺ CD45RO ⁺)	19.3 (12.9 - 22.4)	21.2 (14.4 - 26.8)	NS
BAL Th17 cells (%CD3 ⁺ CD4 ⁺ CD45RO ⁺)	62.7 (58.9 - 68.0)	58.9 (41.7 - 74.2)	NS
BAL CD45RO ⁺ memory T cells (%CD3 ⁺ CD4 ⁺)	82.4 (77.1 - 86.5)	82.2 (64.0 - 87.3)	NS
BAL CD45RA ⁺ naive T cells (%CD3 ⁺ CD4 ⁺)	1.2 (0.2 - 2.1)	1.5 (0.4 - 8.1)	NS
BAL Tregs (%CD3 ⁺ CD4 ⁺)	5.4 (2.1 - 9.4)	3.7 (1.9 - 6.3)	NS
BAL CD3 ⁺ CD4 ⁺ T cells (%CD45 ⁺)	12.0 (6.5 - 38.7)	32.7 (13.1 - 52.5)	NS
BAL CD3 ⁺ CD4 ⁻ T cells (%CD45 ⁺)	26.8 (4.0 - 40.2)	21.0 (17.4 - 35.6)	NS
BAL GM-CSF (pg/mL)	228 (153 - 496)	358 (193 - 571)	NS
BAL IL6 (pg/mL)	60 (42 - 89)	96 (45 - 181)	NS
BAL IL7 (pg/mL)	0.3 (0.3 - 50.4)	0.3 (0.3 - 8.9)	NS
BAL IL8 (pg/mL)	1379 (654 - 1956)	1652 (769 - 3181)	NS
BAL CXCL11 (pg/mL)	365 (155 - 585)	371 (161 - 499)	NS
BAL MIP-1 α (pg/mL)	90 (54 - 155)	79 (36 - 121)	NS
BAL MIP-1 β (pg/mL)	249 (178 - 408)	258 (109 - 339)	NS
BAL MIP-3 α (pg/mL)	316 (105 - 905)	512 (158 - 1583)	NS
BAL TNF (pg/mL)	28 (16 - 63)	64 (14 - 92)	NS
BAL IL-1 β (pg/mL)	0.0 (0.0 - 7.1)	0.0 (0.0 - 1.8)	NS
BAL IL21 (pg/mL)	0.0 (0.0 - 43.9)	0.0 (0.0 - 11.2)	NS

Tables S12: List of specific bacterial taxa that differ in relative abundance in sputum microbiota between Th2-high atopic asthmatic (AA) and healthy control (HC) subjects.

OTU_IDs	Th2-high AAs (mean seq reads)	HC (mean seq reads)	Th2-high AA-HC (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
525942	130.56	21.88	108.68	0.002	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
1004910	177.11	101.31	75.80	2.65E-54	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4440404	66.89	0.81	66.08	1.15E-53	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	
642465	70.33	13.25	57.08	5.38E-96	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
941024	32.44	0.81	31.63	1.14E-36	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
OTU938	20.89	9.00	11.89	0.078	Firmicutes	Bacilli	Lactobacillales			
124932	22.44	15.13	7.32	0.001	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	
OTU1170	10.89	3.75	7.14	4.53E-09	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
949816	5.78	2.50	3.28	0.011	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
OTU231	3.33	0.63	2.71	0.007	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Granulicatella	
4469627	3.00	0.88	2.13	0.005	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	
OTU1512	1.00	4.13	-3.13	0.070	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar
3922	0.33	3.94	-3.60	0.001	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	
95168	2.33	6.00	-3.67	0.002	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	
495451	0.78	5.06	-4.28	7.09E-05	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
610111	0.22	4.69	-4.47	0.020	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
253550	6.11	10.94	-4.83	0.005	Bacteroidetes	Bacteroidia	Bacteroidales			
539819	3.56	8.56	-5.01	0.000	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Megasphaera	
4351304	0.89	6.06	-5.17	7.43E-06	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	
4477971	1.56	6.75	-5.19	9.46E-06	Firmicutes	Clostridia	Clostridiales			
790466	7.89	13.88	-5.99	0.001	Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Anaerovorax	

OTU_IDs	Th2-high AAs (mean seq reads)	HC (mean seq reads)	Th2-high AA-HC (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
55854	0.44	7.25	-6.81	1.81E-06	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4307006	24.67	32.06	-7.40	0.026	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
561935	2.00	11.00	-9.00	3.63E-10	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	
OTU328	11.78	21.13	-9.35	6.62E-06	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
3581175	4.11	14.13	-10.01	2.52E-10	Firmicutes	Clostridia	Clostridiales	Veillonellaceae		
828676	0.44	11.38	-10.93	7.68E-09	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
4380570	2.22	16.81	-14.59	2.01E-16	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Kingella	
239506	28.56	44.25	-15.69	9.36E-08	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU1053	0.11	17.94	-17.83	1.43E-05	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
4294775	1.56	22.38	-20.82	1.11E-20	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	
4404577	82.78	105.44	-22.66	1.78E-06	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Peptostreptococcus	
4412991	0.22	23.50	-23.28	0.044	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae		
851961	13.89	42.38	-28.49	2.01E-28	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
22951	0.22	43.94	-43.72	0.000	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
3678349	9.56	81.06	-71.51	0.032	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus
594026	1.67	91.75	-90.08	9.35E-52	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
252843	12.67	175.00	-162.33	9.97E-164	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
114813	6.44	235.06	-228.62	2.72E-160	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4335450	3.11	391.44	-388.33	2.07E-141	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae		

Tables S13: List of specific bacterial taxa that differ in relative abundance in sputum microbiota between Th2-high atopic asthmatic (AA) and atopic non-asthmatic control (ANA) subjects.

OTU_IDs	Th2-high AAs (mean seq reads)	ANA (mean seq reads)	Th2-high AA-ANA (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
4451251	350.33	102.39	247.94	0.084	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Atopobium	
525942	130.56	39.56	91.00	0.084	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
530206	106.56	39.94	66.61	0.075	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
4440404	66.89	1.56	65.33	1.35E-81	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	
4318122	313.22	278.72	34.50	3.83E-05	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Actinobacillus	porcinus
4426165	46.89	14.44	32.44	2.87E-48	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
941024	32.44	2.11	30.33	3.18E-54	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
855912	28.78	3.22	25.56	4.18E-49	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
124932	22.44	5.83	16.61	4.64E-27	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	
4299448	11.89	1.33	10.56	0.075	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	
OTU900	11.00	5.00	6.00	0.060	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
4458755	6.56	0.78	5.78	0.060	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
OTU84330	2.56	0.44	2.11	0.084	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
4477971	1.56	4.17	-2.61	0.026	Firmicutes	Clostridia	Clostridiales			
OTU41	0.11	3.00	-2.89	0.082	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
OTU95891	0.22	5.67	-5.44	0.007	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
OTU594	0.44	6.39	-5.94	0.037	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	Prevotella	tanneriae
2222	0.44	6.50	-6.06	0.010	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU671	1.11	9.33	-8.22	0.055	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
610111	0.22	11.67	-11.44	0.007	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU1465	0.44	13.83	-13.39	5.73E-10	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	intermedia
250288	1.33	15.67	-14.33	0.091	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae		

OTU_IDs	Th2-high AAs (mean seq reads)	ANA (mean seq reads)	Th2-high AA-ANA (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
OTU517	4.11	21.22	-17.11	0.075	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
4409545	7.33	25.22	-17.89	0.070	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
OTU575	9.11	29.17	-20.06	0.062	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
OTU1635	1.33	22.72	-21.39	6.79E-07	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	nanceiensis
4412630	1.67	23.44	-21.78	0.001	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
OTU328	11.78	36.72	-24.94	0.077	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
4337383	0.56	37.17	-36.61	0.067	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae		
22951	0.22	46.39	-46.17	3.08E-12	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4392350	12.22	63.39	-51.17	0.086	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	
4469627	3.00	74.44	-71.44	6.54E-59	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	
114813	6.44	83.94	-77.50	1.70E-79	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
3678349	9.56	104.11	-94.56	0.060	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus
828676	0.44	103.06	-102.61	1.45E-25	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
594026	1.67	165.89	-164.22	0.013	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
799211	100.22	704.50	-604.28	0.077	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	Prevotella	
4310398	184.78	847.72	-662.94	0.080	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	nanceiensis

Tables S14: List of specific bacterial taxa that differ in relative abundance in sputum microbiota between Th2-high atopic asthmatic (AA) and Th2-low AA subjects.

OTU_IDs	Th2-high AAs (mean seq reads)	Th2-low AAs (mean seq reads)	Th2-high - low AA (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
4466659	59.33	10.64	48.70	0.039	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
855912	28.78	7.95	20.82	5.07E-37	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
4369050	15.56	1.95	13.60	0.050	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
OTU84330	2.56	0.36	2.19	0.021	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
OTU1632	0.44	2.68	-2.24	0.019	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
42538	2.56	5.55	-2.99	0.022	Tenericutes	Mollicutes	RF39			
OTU594	0.44	3.45	-3.01	0.089	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]	tanneriae
4354103	0.89	4.27	-3.38	0.001	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
OTU216	0.11	3.64	-3.53	0.019	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
2222	0.44	4.05	-3.60	0.005	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU255	0.56	5.09	-4.54	7.31E-05	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	
1015518	0.11	5.32	-5.21	0.005	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	
4469627	3.00	9.32	-6.32	2.32E-06	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	
14920	1.44	8.23	-6.78	1.20E-07	Firmicutes	Bacilli	Gemellales	Gemellaceae	Gemella	
OTU554	0.78	7.95	-7.18	0.041	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]	
288521	0.11	7.32	-7.21	0.001	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
4406782	0.78	8.18	-7.40	0.076	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae		
1053321	0.11	7.91	-7.80	0.001	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	
OTU671	1.11	10.14	-9.03	0.056	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU479	0.22	9.32	-9.10	1.02E-05	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	

OTU_IDs	Th2-high AAs (mean seq reads)	Th2-low AAs (mean seq reads)	Th2-high - low AA (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
OTU1508	1.11	10.32	-9.21	0.097	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]	
610111	0.22	9.45	-9.23	0.033	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU1635	1.33	11.14	-9.80	0.016	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	nanceiensis
134726	0.11	10.59	-10.48	0.000	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
1927234	1.67	21.36	-19.70	0.064	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
OTU955	3.89	25.86	-21.97	0.073	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
4430581	3.22	27.45	-24.23	0.050	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]	
4440404	66.89	131.82	-64.93	2.81E-49	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	
246528	8.44	95.09	-86.65	8.73E-93	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	
4307790	41.00	177.59	136.59	0.064	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Selenomonas	

Tables S15: List of specific bacterial taxa that differ in relative abundance in sputum microbiota between Th2-low atopic asthmatic (AA) and healthy control (HC) subjects.

OTU_IDs	Th2-low AAs (mean seq reads)	HC (mean seq reads)	Th2-low AA-HC (mean seq reads)	q- value	Phylum	Class	Order	Family	Genus	Species
968873	151.23	0.81	150.41	0.016	Bacteroidetes	Flavobacteriia	Flavobacteriales	Weeksellaceae		
4318671	239.27	103.31	135.96	0.011	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	dispar
4440404	131.82	0.81	131.01	0.086	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	
4428313	49.68	0.13	49.56	0.043	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
567427	50.68	8.06	42.62	0.015	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
4299448	14.68	1.44	13.24	0.058	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	
240049	13.41	2.56	10.85	0.064	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4469627	9.32	0.88	8.44	1.30E-15	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	
OTU733	8.41	0.13	8.28	2.28E-07	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	
14920	8.23	0.25	7.98	4.26E-10	Firmicutes	Bacilli	Gemellales	Gemellaceae	Gemella	
855912	7.95	0.13	7.83	3.47E-07	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
1053321	7.91	0.25	7.66	6.93E-10	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	
288521	7.32	0.13	7.19	6.67E-07	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
42538	5.55	0.19	5.36	0.026	Tenericutes	Mollicutes	RF39			
4354103	4.27	0.06	4.21	0.001	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
OTU216	3.64	0.19	3.45	2.73E-05	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
63117	1.23	3.81	-2.59	5.01E-05	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Kingella	
851668	0.41	3.88	-3.47	2.22E-08	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4299087	0.77	6.25	-5.48	0.048	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	ochracea
828676	4.73	11.38	-6.65	8.83E-11	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	

OTU_IDs	Th2-low AAs (mean seq reads)	HC (mean seq reads)	Th2-low AA-HC (mean seq reads)	q- value	Phylum	Class	Order	Family	Genus	Species
539819	0.55	8.56	-8.02	7.85E-18	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Megasphaera	
4454385	2.73	13.50	-10.77	0.066	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
526131	2.64	13.88	-11.24	0.026	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
956702	0.14	11.88	-11.74	1.74E-12	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	influenzae
29566	0.91	13.88	-12.97	3.08E-29	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Sneathia	
OTU1595	0.14	13.25	-13.11	0.095	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
790466	0.23	13.88	-13.65	0.018	Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Anaerovorax	
OTU1680	0.09	17.25	-17.16	0.053	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
OTU391	0.64	17.81	-17.18	9.41E-32	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae		
4306356	1.95	22.00	-20.05	0.011	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	
4294775	0.05	22.38	-22.33	0.013	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	
669126	0.18	22.75	-22.57	0.003	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	
4334479	4.32	26.94	-22.62	0.002	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	
4207350	22.32	49.13	-26.81	2.93E-40	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Aggregatibacter	
4344207	14.86	49.75	-34.89	0.058	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus
4466659	10.64	49.50	-38.86	0.008	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
4297695	7.55	50.00	-42.45	0.062	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Campylobacter	
252843	124.18	175.00	-50.82	7.79E-35	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
121566	0.09	62.00	-61.91	4.58E-18	Tenericutes	Mollicutes	Acholeplasmatales	Acholeplasmataceae	Acholeplasma	
3678349	18.09	81.06	-62.97	0.082	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus
4442779	9.82	82.63	-72.81	0.017	Proteobacteria	Gammaproteobacteria	Cardiobacteriales	Cardiobacteriaceae	Cardiobacterium	
114813	0.95	235.06	-234.11	0.040	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4335450	0.23	391.44	-391.21	1.48E-59	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae		

OTU_IDs	Th2-low AAs (mean seq reads)	HC (mean seq reads)	Th2-low AA-HC (mean seq reads)	q- value	Phylum	Class	Order	Family	Genus	Species
4315974	167.36	642.19	-474.82	0.040	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	

Tables S16: List of specific bacterial taxa that differ in relative abundance in sputum microbiota between Th2-low atopic asthmatic (AA) and atopic non-asthmatic control (ANA) subjects.

OTU_IDs	Th2-low AAs (mean seq reads)	ANA (mean seq reads)	Th2-low AA-ANA (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
4440404	131.82	1.56	130.26	0.083	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	
530206	98.91	39.94	58.96	0.024	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
4297119	69.91	17.50	52.41	0.027	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
4440670	45.68	0.11	45.57	0.001	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	
567427	50.68	7.39	43.29	0.004	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
521268	50.05	16.83	33.21	0.032	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
70628	25.05	2.33	22.71	0.008	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
4321397	24.36	9.83	14.53	0.072	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
4299448	14.68	1.33	13.35	0.027	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	
OTU1352	15.05	2.17	12.88	4.29E-28	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	
886735	12.64	0.11	12.53	2.06E-09	Firmicutes	Bacilli	Lactobacillales	Aerococcaceae	Alloiococcus	
923032	12.23	0.44	11.78	0.035	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
OTU479	9.32	0.44	8.87	4.05E-15	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	
4207350	22.32	13.56	8.76	1.28E-08	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Aggregatibacter	
1033013	8.50	0.17	8.33	1.24E-09	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
OTU66	7.14	0.11	7.03	2.88E-07	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
288521	7.32	0.56	6.76	2.99E-13	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
3581175	6.05	0.11	5.93	0.019	Firmicutes	Clostridia	Clostridiales	Veillonellaceae		
14920	8.23	2.89	5.34	2.18E-09	Firmicutes	Bacilli	Gemellales	Gemellaceae	Gemella	
OTU900	10.05	5.00	5.05	0.036	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
1015518	5.32	0.50	4.82	7.85E-10	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	
855912	7.95	3.22	4.73	1.52E-07	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	

OTU_IDs	Th2-low AAs (mean seq reads)	ANA (mean seq reads)	Th2-low AA-ANA (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
OTU1366	5.45	0.78	4.68	5.60E-10	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	parainfluenzae
4354103	4.27	0.17	4.11	1.76E-06	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	
OTU216	3.64	0.11	3.53	5.80E-05	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
3130888	6.77	3.39	3.38	0.000	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	parainfluenzae
OTU1207	5.09	2.61	2.48	0.062	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
3931	0.27	2.28	-2.01	6.01E-05	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	
OTU9589 1	1.45	5.67	-4.21	0.02346 31	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
OTU1465	4.27	13.83	-9.56	4.33E-20	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	intermedia
677680	1.77	11.89	-10.12	0.098	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	Prevotella	
4310208	4.59	17.00	-12.41	0.044	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	parvula
526131	2.64	16.00	-13.36	0.008	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	
4412630	5.05	23.44	-18.40	0.008	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
1053321	7.91	26.33	-18.42	1.28E-39	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	
4466659	10.64	39.56	-28.92	0.013	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
4330572	17.59	52.33	-34.74	0.094	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	
4337383	1.27	37.17	-35.89	0.023	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae		
2654263	18.27	55.61	-37.34	0.099	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	parainfluenzae
4344207	14.86	59.00	-44.14	0.099	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus
2613485	4.05	49.33	-45.29	1.67E-109	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
964220	19.27	72.00	-52.73	0.027	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	durum
4469627	9.32	74.44	-65.13	2.87E-166	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	
114813	0.95	83.94	-82.99	7.55E-90	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
3678349	18.09	104.11	-86.02	0.041	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	anginosus

OTU_IDs	Th2-low AAs (mean seq reads)	ANA (mean seq reads)	Th2-low AA-ANA (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
828676	4.73	103.06	-98.33	2.13E- 202	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	
4428060	42.68	236.56	-193.87	0.007	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica

Table S17: Clinical and inflammatory characteristics of Th2-low asthmatics based on distinct airway inflammatory cytokine clusters (Benjamini-Hochberg adjusted p-value).

Variable	ICC1 (n=5)	ICC2 (n=18)	q-value
Age (yrs)	48 (36 - 48)	27 (21 - 35)	NS
ACQ Score (Baseline)*	0.3 (0.1 - 1.0)	0.4 (0.0 - 0.7)	NS
Age asthma diagnosed (yrs)	23 (6 - 36)	9 (4 - 16)	NS
% Male	20%	44%	NS
% White	60%	56%	NS
BMI	26 (23 - 33)	26 (23 - 29)	NS
FEV1 % predicted pre-Ab [‡]	95 (82 - 102)	86 (76 - 101)	NS
FEV1 % predicted post-Ab [‡]	105 (89 - 114)	97 (84 - 106)	NS
Change in FEV1%	7 (7 - 14)	7 (5 - 13)	NS
PC ₂₀ (methacholine)	0.6 (0.3 - 4.1)	1.9 (0.9 - 6.0)	NS
Serum IgE (EU/mL)	186 (102 - 226)	97 (33 - 213)	NS
No. of positive sIgE [‡]	6 (5 - 8)	3 (2 - 7)	NS
Atopic rhinitis (%) [§]	60%	56%	NS
Eczema/Atopic dermatitis (%) [§]	40%	39%	NS
Blood neutrophils (%)	53 (48 - 60)	55 (48 - 61)	NS
Blood eosinophils (absolute)	200 (150 - 619)	130 (100 - 238)	NS
Blood eosinophils (%)	5 (3 - 8)	3 (1 - 4)	NS
Sputum neutrophils (%)	33 (22 - 69)	56 (36 - 65)	NS
Sputum eosinophils (%)	0.4 (0.2 - 9.9)	0.2 (0.0 - 0.9)	NS
TGM [§] score	0.3 (-0.3 - 0.8)	0.2 (-0.2 - 0.5)	NS
Blood Th1 (%CD45+)	4.0 (3.1 - 4.9)	4.0 (2.0 - 5.5)	NS
Blood Th2 (%CD45+)	3.3 (2.8 - 5.0)	4.2 (2.5 - 5.5)	NS
Blood Th17 (%CD45+)	6.8 (5.5 - 8.7)	8.2 (5.8 - 11.1)	NS
Blood neutrophils (%CD45+)	0.9 (0.2 - 11.7)	0.2 (0.1 - 0.6)	NS
Blood eosinophils (%CD45+)	2.5 (0.5 - 2.9)	0.8 (0.4 - 2.6)	NS
Blood basophils (%CD45+)	1.0 (0.9 - 1.3)	0.8 (0.6 - 1.3)	NS
Blood pDC (%CD45+)	0.2 (0.1 - 0.9)	0.3 (0.2 - 0.5)	NS
Blood Th1 cells (%CD3+CD4+CD45RO+)	31.4 (19.9 - 32.5)	23.5 (17.7 - 31.3)	NS
Blood Th2 cells (%CD3+CD4+CD45RO+)	21.7 (21.5 - 31.8)	26.0 (21.2 - 31.5)	NS
Blood Th17 cells (%CD3+CD4+CD45RO+)	46.9 (44.7 - 49.8)	47.4 (39.0 - 60.4)	NS
Blood CD45RO+ memory T cells (%CD3+CD4+)	34.8 (34.4 - 47.4)	44.2 (36.3 - 55.9)	NS
Blood CD45RA+ naive T cells (%CD3+CD4+)	49.6 (29.9 - 51.9)	38.4 (23.5 - 47.3)	NS
Blood Tregs (%CD3+CD4+)	4.8 (4.3 - 6.0)	4.1 (2.9 - 4.8)	NS

Variable	ICC1 (n=5)	ICC2 (n=18)	q-value
Blood CD3 ⁺ CD4 ⁺ T cells (%CD45 ⁺)	36.4 (32.0 - 44.1)	35.8 (32.9 - 39.9)	NS
Blood CD3 ⁺ CD4 ⁻ T cells (%CD45 ⁺)	26.3 (20.3 - 31.6)	29.5 (23.4 - 33.0)	NS
BAL Th1 (%CD45+)	1.3 (0.3 - 8.5)	3.8 (1.9 - 5.6)	NS
BAL Th2 (%CD45+)	1.6 (0.5 - 6.8)	4.1 (1.9 - 8.3)	NS
BAL Th17 (%CD45+)	4.9 (0.7 - 24.6)	13.0 (6.5 - 23.2)	NS
BAL neutrophils (%CD45+)	0.1 (0.1 - 0.3)	0.1 (0.0 - 0.3)	NS
BAL eosinophils (%CD45+)	0.1 (0.0 - 0.3)	0.1 (0.0 - 0.8)	NS
BAL basophils (%CD45+)	0.1 (0.0 - 0.1)	0.0 (0.0 - 0.1)	NS
BAL pDC (%CD45+)	0.3 (0.1 - 0.9)	0.5 (0.2 - 1.1)	NS
BAL Th1 cells (%CD3 ⁺ CD4 ⁺ CD45RO ⁺)	17.9 (16.7 - 23.7)	15.9 (10.9 - 20.7)	NS
BAL Th2 cells (%CD3 ⁺ CD4 ⁺ CD45RO ⁺)	19.5 (17.0 - 30.9)	21.9 (11.6 - 26.8)	NS
BAL Th17 cells (%CD3 ⁺ CD4 ⁺ CD45RO ⁺)	59.5 (49.4 - 64.2)	58.9 (40.2 - 75.2)	NS
BAL CD45RO ⁺ memory T cells (%CD3 ⁺ CD4 ⁺)	81.6 (51.3 - 87.2)	81.2 (64.0 - 87.3)	NS
BAL CD45RA ⁺ naive T cells (%CD3 ⁺ CD4 ⁺)	0.9 (0.3 - 28.2)	1.5 (0.5 - 8.1)	NS
BAL Tregs (%CD3 ⁺ CD4 ⁺)	3.3 (2.3 - 5.1)	4.1 (1.5 - 6.6)	NS
BAL CD3 ⁺ CD4 ⁺ T cells (%CD45 ⁺)	8.6 (3.2 - 47.6)	32.7 (21.4 - 52.5)	NS
BAL CD3 ⁺ CD4 ⁻ T cells (%CD45 ⁺)	19.2 (4.2 - 43.5)	21.0 (17.4 - 35.6)	NS
BAL GM-CSF (pg/mL)	521 (377 - 1694)	339 (178 - 571)	NS
BAL IL6 (pg/mL)	406 (162 - 419)	77 (25 - 143)	0.076
BAL IL7 (pg/mL)	54.3 (17.9 - 78.8)	0.3 (0.3 - 0.3)	0.020
BAL IL8 (pg/mL)	5424 (4651 - 4796)	1271 (606 - 1804)	0.006
BAL CXCL11 (pg/mL)	352 (152 - 1109)	378 (169 - 499)	NS
BAL MIP-1 α (pg/mL)	254 (88 - 297)	72 (29 - 95)	NS
BAL MIP-1 β (pg/mL)	667 (351 - 1277)	240 (108 - 328)	NS
BAL MIP-3 α (pg/mL)	3482 (1594 - 3632)	390 (151 - 1159)	0.020
BAL TNF (pg/mL)	87 (39 - 209)	57 (14 - 81)	NS
BAL IL-1 β (pg/mL)	0.0 (0.0 - 16.2)	0.0 (0.0 - 1.1)	NS
BAL IL21 (pg/mL)	11.2 (5.6 - 84.2)	0.0 (0.0 - 5.2)	NS

Table S18: Clinical and inflammatory characteristics of ICS-responders and non-responders.

Variable	ICSNR (n=8)	ICSR (n=12)	q-value
Age (yrs)	30 (25 - 40)	33 (27 - 43)	NS
ACQ Score (Baseline)*	0.4 (0.0 - 1.2)	0.6 (0.0 - 0.6)	NS
Age asthma diagnosed (yrs)	5 (1 - 9)	9 (6 - 21)	NS
% Male	38%	33%	NS
% White	63%	33%	NS
BMI	26 (24 - 29)	27 (24 - 30)	NS
FEV1 % predicted pre-Ab [‡]	101 (95 - 113)	84 (71 - 95)	0.062
FEV1 % predicted post-Ab [‡]	106 (101 - 115)	99 (86 - 104)	NS
Change in FEV%	5 (4 - 6)	13 (7 - 17)	0.062
PC ₂₀ (methacholine)	1.9 (0.6 - 4.4)	0.6 (0.2 - 1.0)	NS
Change in PC20 (Log2)	0.3 (-0.7 - 0.6)	2.2 (1.3 - 3.7)	0.006
Serum IgE (EU/mL)	197 (100 - 316)	139 (80 - 311)	NS
No. of positive sIgE [‡]	6 (2 - 6)	5 (2 - 9)	NS
Atopic rhinitis (%) [§]	63%	50%	NS
Eczema/Atopic dermatitis (%) [§]	25%	67%	NS
Blood neutrophils (%)	58 (53 - 65)	53 (50 - 59)	NS
Blood eosinophils (absolute)	200 (100 - 345)	210 (93 - 475)	NS
Blood eosinophils (%)	3 (1 - 6)	4 (2 - 7)	NS
Sputum neutrophils (%)	59 (42 - 67)	52 (25 - 66)	NS
Sputum eosinophils (%)	0.4 (0.0 - 0.7)	0.1 (0.0 - 0.7)	NS
TGM\$ score	0.5 (-0.7 - 1.1)	0.5 (0.2 - 1.5)	NS
Blood Th1 (%CD45+)	4.2 (1.5 - 5.4)	4.3 (3.2 - 5.6)	NS
Blood Th2 (%CD45+)	4.3 (2.3 - 6.0)	4.2 (2.9 - 5.1)	NS
Blood Th17 (%CD45+)	8.1 (4.4 - 10.5)	7.3 (5.2 - 10.6)	NS
Blood neutrophils (%CD45+)	0.2 (0.0 - 1.1)	0.2 (0.1 - 0.4)	NS
Blood eosinophils (%CD45+)	0.5 (1.1 - 2.5)	2.3 (0.4 - 7.9)	NS
Blood basophils (%CD45+)	0.6 (0.1 - 1.3)	1.2 (0.6 - 1.4)	NS
Blood pDC (%CD45+)	0.1 (0.0 - 0.3)	0.4 (0.3 - 0.7)	0.078
Blood Th1 cells (%CD3+CD4+CD45RO+)	22.1 (16.0 - 33.4)	30.0 (22.2 - 34.0)	NS
Blood Th2 cells (%CD3+CD4+CD45RO+)	24.8 (15.3 - 30.1)	25.7 (21.8 - 31.1)	NS
Blood Th17 cells (%CD3+CD4+CD45RO+)	49.3 (39.1 - 63.2)	46.5 (39.8 - 49.4)	NS
Blood CD45RO ⁺ memory T cells (%CD3+CD4 ⁺)	40.3 (34.9 - 55.7)	38.9 (34.7 - 54.4)	NS
Blood CD45RA ⁺ naive T cells (%CD3+CD4 ⁺)	44.3 (26.5 - 50.9)	44.1 (28.2 - 49.7)	NS
Blood Tregs (%CD3+CD4 ⁺)	4.2 (2.5 - 5.7)	4.3 (3.1 - 5.9)	NS
Blood CD3 ⁺ CD4 ⁺ T cells (%CD45 ⁺)	39.0 (34.8 - 44.6)	38.2 (35.2 - 43.0)	NS
Blood CD3 ⁺ CD4 ⁻ T cells (%CD45 ⁺)	29.1 (20.6 - 34.6)	29.8 (26.0 - 32.2)	NS

Variable	ICSNR (n=8)	ICSR (n=12)	q-value
BAL Th1 (%CD45+)	1.6 (0.4 - 3.4)	3.1 (1.4 - 5.8)	NS
BAL Th2 (%CD45+)	1.7 (0.6 - 4.9)	2.8 (1.7 - 7.8)	NS
BAL Th17 (%CD45+)	5.1 (1.3 - 9.6)	13.0 (5.8 - 21.1)	NS
BAL neutrophils (%CD45+)	0.2 (0.1 - 2.7)	0.1 (0.0 - 0.4)	NS
BAL eosinophils (%CD45+)	0.6 (0.1 - 0.9)	0.1 (0.0 - 0.7)	NS
BAL basophils (%CD45+)	0.1 (0.0 - 0.2)	0.0 (0.0 - 0.1)	NS
BAL pDC (%CD45+)	0.2 (0.0 - 0.5)	0.6 (0.2 - 1.0)	NS
BAL Th1 cells (%CD3+CD4+CD45RO+)	16.5 (13.8 - 19.3)	19.1 (14.5 - 21.2)	NS
BAL Th2 cells (%CD3+CD4+CD45RO+)	20.5 (13.3 - 28.2)	22.3 (13.4 - 26.0)	NS
BAL Th17 cells (%CD3+CD4+CD45RO+)	61.0 (49.9 - 74.9)	59.4 (54.1 - 66.2)	NS
BAL CD45RO ⁺ memory T cells (%CD3 ⁺ CD4 ⁺)	79.9 (57.5 - 84.4)	80.8 (64.2 - 85.0)	NS
BAL CD45RA ⁺ naive T cells (%CD3 ⁺ CD4 ⁺)	1.8 (0.4 - 10.3)	2.0 (1.1 - 9.1)	NS
BAL Tregs (%CD3 ⁺ CD4 ⁺)	4.7 (2.4 - 6.2)	3.5 (1.7 - 8.8)	NS
BAL CD3 ⁺ CD4 ⁺ T cells (%CD45 ⁺)	11.1 (4.0 - 23.4)	30.1 (13.8 - 41.1)	NS
BAL CD3 ⁺ CD4 ⁻ T cells (%CD45 ⁺)	13.8 (4.6 - 47.1)	24.4 (16.5 - 37.8)	NS
BAL GM-CSF (pg/mL)	344 (157 - 559)	335 (185 - 628)	NS
BAL IL6 (pg/mL)	68 (56 - 126)	79 (27 - 118)	NS
BAL IL7 (pg/mL)	0.3 (0.3 - 0.3)	0.3 (0.3 - 19.2)	NS
BAL IL8 (pg/mL)	1747 (1116 - 3871)	1242 (697 - 2688)	NS
BAL CXCL11 (pg/mL)	179 (145 - 372)	390 (145 - 733)	NS
BAL MIP-1 α (pg/mL)	49 (31 - 92)	78 (30 - 155)	NS
BAL MIP-1 β (pg/mL)	220 (98 - 351)	230 (115 - 315)	NS
BAL MIP-3 α (pg/mL)	714 (334 - 3143)	173 (54 - 674)	NS
BAL TNF (pg/mL)	32 (2 - 93)	35 (10 - 77)	NS
BAL IL-1 β (pg/mL)	0.0 (0.0 - 3.8)	0.0 (0.0 - 7.5)	NS
BAL IL21 (pg/mL)	0.0 (0.0 - 0.6)	0.0 (0.0 - 45.1)	NS

Tables S19: Mantel Statistics for correlation in IS and OW microbiota composition of AAs pre- and post- treatment with ICS or placebo.

	Sputum microbiota		Oral microbiota	
	r	p-value	r	p-value
Placebo pre- vs. post-	0.42	0.06	0.84	0.01
ICSR pre- vs. post-	0.59	0.01	0.63	0.01
ICSNR pre- vs. post-	0.23	0.32	0.34	0.11

Tables S20: Specific taxa differentially expressed in the sputum microbiota of ICS non-responders (ICSNR) compared to ICS responders (ICSR) at baseline.

OTU_IDs	ICSNR (mean seq reads)	ICSR (mean seq reads)	ICSNR- ICSR (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
4428313	130.25	3.92	126.33	4.15E-119	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	-
567427	124.00	9.50	114.50	0.019	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	-
2438396	69.00	9.75	59.25	7.48E-80	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	-
923032	35.38	3.67	31.71	3.73E-42	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	-
941024	35.25	6.00	29.25	8.87E-39	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	-
855912	35.63	7.67	27.96	1.79E-35	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	-
124932	20.00	5.83	14.17	8.15E-16	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Treponema	-
63117	7.38	3.67	3.71	0.018	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Kingella	-
1010329	0.38	3.08	-2.71	0.018	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	-
251967	0.38	3.08	-2.71	0.018	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	-
1044611	1.13	4.75	-3.63	0.003	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	-	-
253550	0.25	4.67	-4.42	0.002	Bacteroidetes	Bacteroidia	Bacteroidales	-	-	-
245523	0.25	4.83	-4.58	0.002	Bacteroidetes	Bacteroidia	Bacteroidales	-	-	-
114813	0.38	6.25	-5.88	9.86E-05	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	-

OTU_IDs	ICSNR (mean seq reads)	ICSR (mean seq reads)	ICSNR- ICSNR (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
828676	0.25	8.17	-7.92	6.24E-05	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Fusobacterium	-
851961	0.88	10.58	-9.71	1.13E-08	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	-
OTU479	0.13	17.17	-17.04	5.70E-05	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	-
4317477	0.50	19.08	-18.58	0.046	Actinobacteria	Actinobacteria	Actinomycetales	Actinomycetaceae	Actinomyces	-
4418210	0.13	19.83	-19.71	2.86E-05	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter	-
71957	0.13	29.17	-29.04	3.72E-06	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	-
269907	319.38	354.75	-35.38	0.001	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]	-
4373910	11.00	46.83	-35.83	1.48E-34	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	Neisseria	-
4372058	3.25	56.42	-53.17	0.081	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	melaninogenica
1004910	109.75	183.17	-73.42	1.63E-35	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	-
4377418	12.63	111.58	-98.96	0.023	Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Parvimonas	-
4404577	68.13	177.75	-109.63	4.04E-86	Firmicutes	Clostridia	Clostridiales	Peptostreptococcac eae	Peptostreptococcus	-
2613485	0.50	563.50	-563.00	1.92E-42	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadace ae	Porphyromonas	-

Tables S21: Specific taxa differentially expressed in the oral microbiota of ICS non-responders (ICSNR) compared to ICS responders (ICSR) at baseline.

OTU_IDs	ICSNR (mean seq reads)	ICSR (mean seq reads)	ICSNR- ICSR (mean seq reads)	q-value	Phylum	Class	Order	Family	Genus	Species
4440670	43.50	3.77	39.73	3.87E-55	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	
4445508	9.38	1.23	8.14	3.25E-11	Firmicutes	Clostridia	Clostridiales	Veillonellaceae	Veillonella	
677680	3.50	0.85	2.65	0.005	Bacteroidetes	Bacteroidia	Bacteroidales	[Paraprevotellaceae]	[Prevotella]	
OTU22287	0.25	2.77	-2.52	0.065	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	
4307230	0.25	4.46	-4.21	0.005	Synergistetes	Synergistia	Synergistales	Dethiosulfovibrionaceae	TG5	
114813	0.25	5.31	-5.06	0.002	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
4468500	0.88	9.85	-8.97	6.24E-08	Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	Anaerovorax	
1044611	0.63	13.31	-12.68	2.54E-09	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae		
4310024	6.63	23.85	-17.22	1.68E-15	Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Peptococcus	
642465	1.38	26.46	-25.09	1.52E-19	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	
71957	0.13	35.00	-34.88	1.97E-06	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Capnocytophaga	
2613485	0.13	108.23	-108.11	0.051	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	
252843	67.25	193.62	-126.37	8.31E-107	Fusobacteria	Fusobacteriia	Fusobacteriales	Leptotrichiaceae	Leptotrichia	

Supplemental Figures:

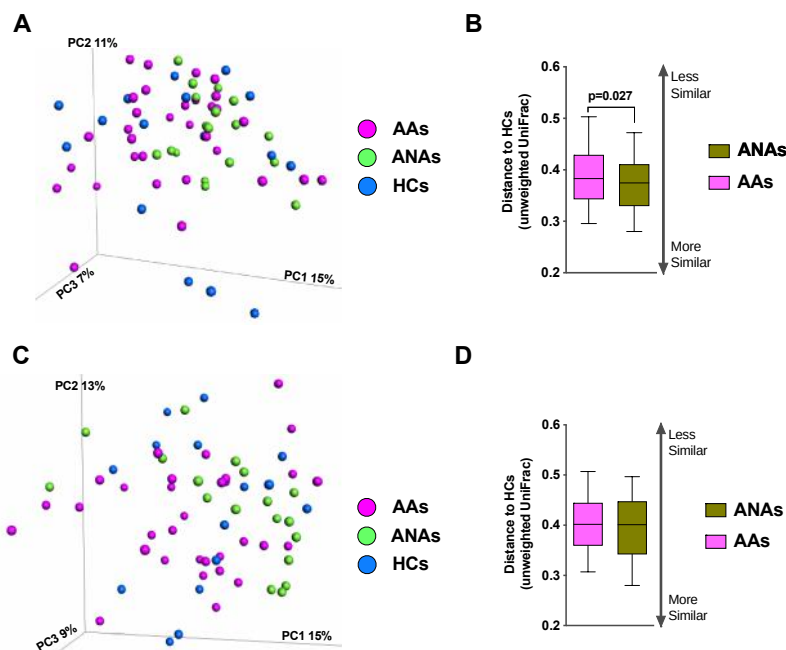


Figure S1: **A.** PCoA plot of IS samples, where each dot represents a sample colored by group. **B.** Sputum microbiota in asthmatic (AA) subjects is more dissimilar from healthy controls (HCs) than the microbiota of allergic non-asthmatic (ANA) subjects. **C.** PCoA plot of OW samples, where each dot represents a sample colored by group. **D.** Oral microbiota composition of the two atopic groups (AA and ANA) is equally dissimilar to that of HC subjects. (Unweighted UniFrac distance; Bonferroni corrected t-test; Whiskers extend to 95% confidence interval).

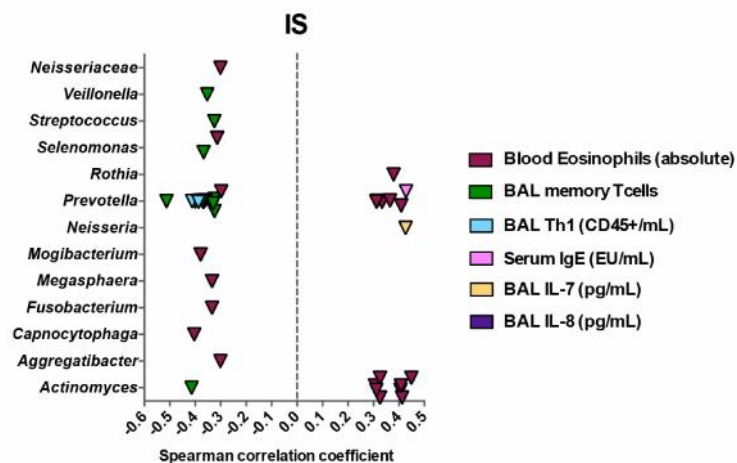


Figure S2: A number of taxa in induced sputum correlated significantly ($q < 0.2$) with specific immunologic markers. Each triangle represents an OTU within a given bacterial genus.

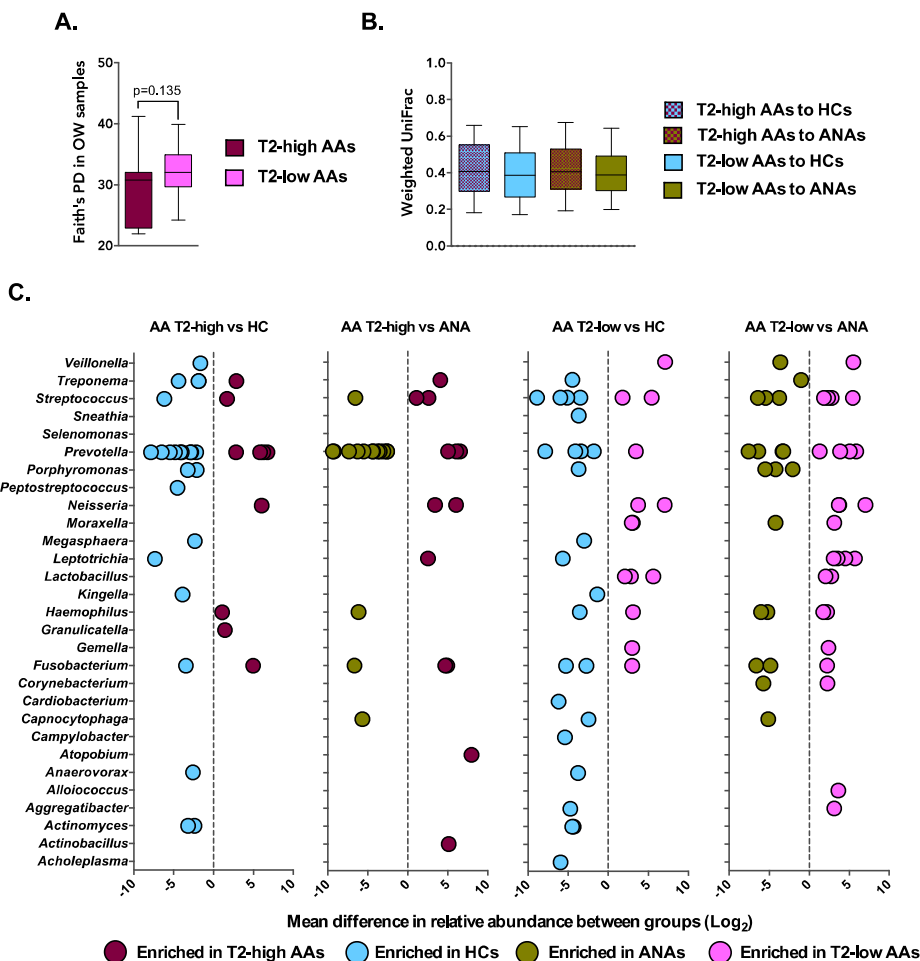


Figure S3: A. Faith's Phylogenetic Diversity (PD) in OW samples of T2-high or -low AA subjects. Statistical significance was determined using Mann-Whitney test. **B.** Comparison of bacterial composition (Unweighted UniFrac) in OW of T2-high and T2-low AAs to that of non-asthmatic controls. Significance was determined using Bonferroni corrected t-test. **C.** Mean difference in relative abundance of specific bacterial taxa present in IS of subjects in the two-group comparison. Each dot represents a unique OTU within a given bacterial genus. Significance was determined using Negative Binomial regression model $q < 0.1$.

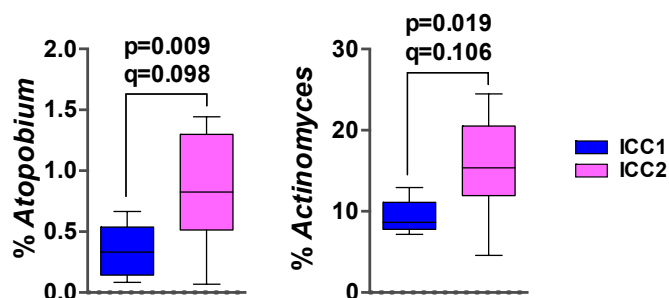


Figure S4: Relative abundance of bacterial genera in oral wash which differentiated ICC1 (blue) and ICC2 (pink) T2-low AA subjects. Statistical significance was determined using Mann-Whitney test, corrected for false discovery.

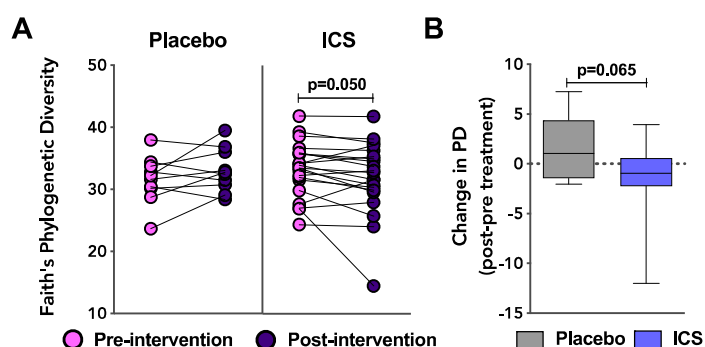


Figure S5: A. Faith Phylogenetic diversity in induced sputum (IS) of ICS-treated asthmatic subjects (n=21) changed with treatment compared to placebo-treated asthmatic subjects (n=10; Wilcoxon matched-pairs signed rank test). **B.** Compared to placebo controls, phylogenetic diversity in IS microbiota trended to decrease following treatment (Mann-Whitney test).

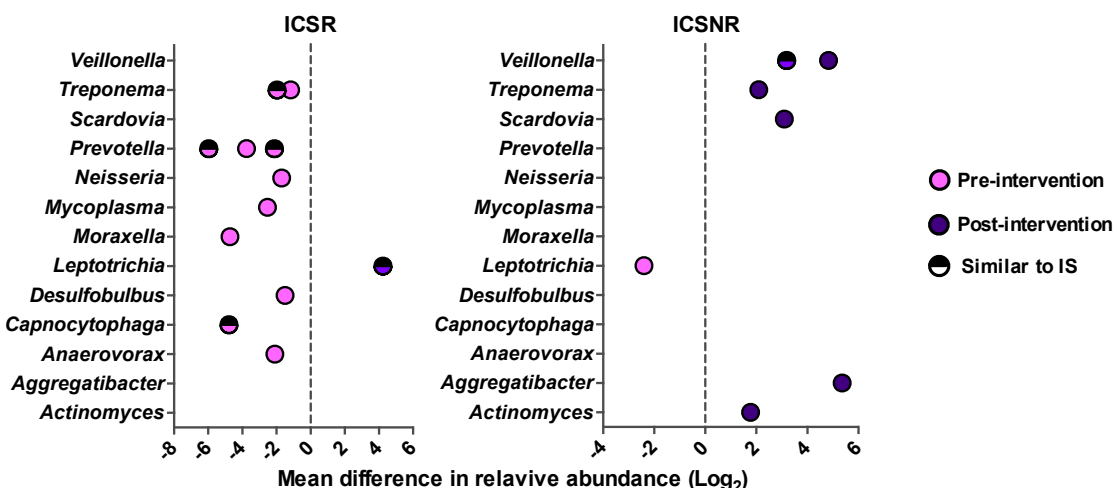


Figure S6: Mean difference in relative abundance of specific bacterial taxa present in OW of ICS-responders (ICSR; n=12) and ICS-non-responders (ICSNR; n=8) pre- and post-intervention with ICS. Each dot represents a unique OTU within a given bacterial genus. Significance was determined using Negative Binomial regression model $q < 0.1$.

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