Airway Microbiota and Bronchial Hyperresponsiveness in Patients with Sub-optimally Controlled Asthma

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Abstract

**Background**—Improvement in lung function following macrolide antibiotic therapy has been attributed to reduction in bronchial infection due to specific bacteria. However, the airway may be populated by a more diverse microbiota, and clinical features of asthma may be associated with characteristics of the airway microbiota present.

**Objective**—To determine if relationships exist between the composition of the airway bacterial microbiota and clinical features of asthma, using culture-independent tools capable of detecting the presence and relative abundance of most known bacteria.

**Methods**—In this pilot study, bronchial epithelial brushings were collected from sixty-five adults with sub-optimally controlled asthma participating in a multicenter study of the effects of clarithromycin on asthma control, and ten healthy subjects. A combination of high-density 16S rRNA microarray and parallel clone library-sequencing analysis was used to profile the microbiota and examine relationships with clinical measurements.

**Results**—Compared to controls, 16S rRNA amplicon concentrations (a proxy for bacterial burden) and bacterial diversity were significantly higher among asthmatic patients. In multivariate analyses, airway microbiota composition and diversity were significantly correlated with bronchial hyperresponsiveness. Specifically, the relative abundance of particular phylotypes, including members of the Comamonadaceae, Sphingomonadaceae, Oxalobacteraceae and other bacterial families, were highly correlated with the degree of bronchial hyperresponsiveness.

**Conclusion**—The composition of bronchial airway microbiota is associated with the degree of bronchial hyperresponsiveness among patients with sub-optimally controlled asthma. These findings support the need for further functional studies to examine the potential contribution of members of the airway microbiota in asthma pathogenesis.

**Keywords**

microbiome; bacteria; asthma; 16S rRNA; PhyloChip

Introduction

Interest in the potential role of bacterial colonization or infection of the bronchial mucosa in the pathogenesis of asthma has been raised by several recent reports: associations between bacterial colonization of neonatal airways and subsequent asthma development\(^1\), evidence of bronchial infection by specific intracellular bacteria among adult asthmatics\(^2\), and the efficacy of prolonged macrolide antibiotic treatment in subsets of asthmatic patients\(^3\)–\(^5\). While the role of microbial infection in asthma pathogenesis remains unclear, studies to date have primarily applied culture-based or targeted molecular approaches to identify specific bacterial species of interest in the airways. However, recent developments in defining the human microbiome have demonstrated that the composition of bacterial communities colonizing mucosal surfaces, rather than simply the presence of individual species, can be important in defining states of health or disease\(^6\)–\(^11\).

Culture-independent microbiota profiling based on sequence polymorphisms in the 16S rRNA gene, present in all bacteria, has been widely applied in environmental ecological studies\(^12\)–\(^14\), and in studies of the human ‘superorganism’\(^9\)–\(^11\). 16S rRNA-based phylogenetic analysis via sequencing or phylogenetic arrays, permits detection of uncultured or fastidious bacteria, providing information on community composition without a priori knowledge of those present\(^15\)–\(^17\). One such platform, the 16S rRNA PhyloChip, has been applied in a number of environmental\(^12\), \(^14\), \(^15\), \(^18\) and clinical studies\(^6,19,20\). This high-density array contains ~500,000 probes that can differentiate ~8,500 bacterial taxa (defined...
as groups of organisms sharing ≥ 97% 16S rRNA sequence homology)\textsuperscript{15, 21} and demonstrates greater resolution of complex bacterial communities than traditional 16S rRNA clone library-sequencing approaches\textsuperscript{6, 18, 21}. It provides an ideal tool to examine relationships between microbiota composition, including the presence and relative abundance of members of the ‘rare biosphere’\textsuperscript{22}, and clinical characteristics of disease.

Recognizing the frequency with which the tracheobronchial tree is exposed to the external environment and to secretions from the oropharynx or upper gastrointestinal tract, we hypothesized that complex bacterial communities may colonize asthmatic airway mucosa and exhibit relationships with clinical features of disease. Therefore, in conjunction with a prospective study of the effects of extended clarithromycin therapy in adults with sub-optimally controlled asthma\textsuperscript{23}, we conducted a pilot study of the airway microbiota in sixty-five adult asthmatic and ten healthy subjects using the PhyloChip and parallel clone library-sequencing. Bronchial airway samples from asthmatics possessed greater bacterial burden and diversity than healthy individuals. Furthermore, the degree of bronchial hyperresponsiveness exhibited by subjects was related to community composition and the relative abundance of specific bacterial families comprising the airway microbiota. Portions of these results have previously been presented in abstract form\textsuperscript{24, 25}.

Materials and Methods

For supplemental methods, please see the Online Repository.

Subjects

Bronchial epithelial samples for microbial analysis were obtained from a subset of subjects enrolled in the Macrolides in Asthma (MIA) study\textsuperscript{23} (NCT00318708, clinicaltrials.gov) conducted by the NHLBI-sponsored Asthma Clinical Research Network. Briefly, adults with clinically stable but sub-optimally controlled asthma, defined as persistent symptoms on Asthma Control Questionnaire\textsuperscript{26} after four weeks of standardized treatment with inhaled fluticasone, were studied. Relevant inclusion/exclusion criteria and study procedures are described in the supplemental methods, including exclusion for symptoms of respiratory infection or asthma exacerbation within 6 weeks of the bronchoscopy visit. Bronchoscopy was performed prior to randomization to clarithromycin or placebo therapy. Three protected specimen bronchial brushings for microbiome analysis were obtained and sent to the University of California, San Francisco, where ten healthy, non-smoking, non-atopic adults without asthma were also enrolled. All protocols were approved by the Institutional Review Board at each center.

Sample processing

All samples first underwent screening by PCR for presence of the 16S rRNA gene, using extracted total DNA (100 ng) and the universal 16S rRNA primers, Bact-27F and Bact-1492R\textsuperscript{27} in a 40-cycle reaction. Samples were deemed positive or negative for bacteria based on presence or absence of a visible 16S rRNA PCR product. Samples with any evidence of a 16S rRNA PCR product were subsequently subjected to eight-PCR reactions (per sample) using the same primer set across a temperature gradient (48–58 °C) to maximize bacterial diversity captured. Amplicons from each sample were then pooled, purified, and gel-quantified using E-gels\textsuperscript{®} (Invitrogen) prior to hybridization of a standardized quantity to the array for each sample, as previously described\textsuperscript{6, 15}. 
Quantitative PCR (Q-PCR) analysis

Q-PCR was performed for twenty-one samples to determine total 16S rRNA copy number, normalized to β-actin copy numbers. Further details are provided in the supplemental material.

Data analysis

Relationships between microbiota structure and study variables were analyzed using multivariate statistics in combination with the Multi-response Permutation Procedure and Indicator Species Analysis (MRPP) and non-metric multi-dimensional scaling (NMDS) ordination. Data were log-transformed prior to analysis where appropriate. Sorensen distance-based dissimilarity matrices were calculated and used for subsequent NMDS analysis to examine the relatedness of samples based on their microbiota composition. Three asthmatic samples with mean relative Sorensen distances greater than two deviations from the pooled mean were excluded for this analysis. Pearson’s correlations were performed to determine relationships between the relative abundance of all detected phylotypes and study variables with q-value corrections for false discovery. Significance was conservatively based on a false discovery rate of < 3% and a probability of no more than two taxa being falsely identified as significant for each test. Community diversity was estimated using Shannon indices, based on the number of taxa present (community “richness”) and their relative abundance (community “evenness”). Statistical analyses were conducted using PC-ORD v5, JMP v7 (SAS Institute, 2007) and R software v.2.7.1.

16S rRNA clone library-sequencing

Clone libraries for a subset of patients were constructed (using the same 16S rRNA amplicon pool hybridized to the array) and sequenced at the Department of Energy, Joint Genome Institute (299–374 clones per library). Quality filtered sequences were aligned and chimera-checked using NAST and Bellerophon, respectively, then classified by ‘G2-chip’ taxonomy.

Phylogenetic tree construction

Representative 16S rRNA sequences for taxa of interest were exported from the Greengenes database. A neighbor-joining tree with nearest-neighbor interchange was produced using FastTree. Trees were annotated using the Interactive Tree of Life.

Results

Airway bacterial burden

Bronchial brushings were obtained from a total of 75 individuals, 65 asthmatic and 10 healthy controls. Subject characteristics are summarized in Table I. All asthmatics fulfilled entry criteria for the parent MIA trial, and samples for this pilot study were processed as they were received with no additional selection criteria applied. On initial screening, 54 of 65 (83%) asthmatics and 8 of 10 (80%) controls exhibited a visible 16S rRNA PCR product. However, not all PCR-positive samples subsequently produced sufficient 16S rRNA amplicon for PhylomChip analysis. While very small amplicon concentrations may be hybridized to the array, in our experience, application of 250 ng provides good characterization of the respiratory microbiota. A greater proportion of asthmatic (56.9%) than healthy subjects (30%) produced 250 ng of amplicon, although this difference was not significant (p = 0.17). To increase the number of samples for comparative analyses, we analyzed the community composition detected by PhylomChip in six paired samples using both 100 ng and 250 ng of amplicon. Finding no significant difference in the
community composition detected in paired samples \( (p_{MRPP} > 0.1) \), seven additional subjects (5 asthmatic, 2 healthy) were assayed by PhyloChip using 100 ng of amplicon. Therefore, array data from a total of 47 subjects (42 asthmatic, 5 healthy) was obtained for the subsequent analyses (See Figure E1 in the Online Repository).

To confirm that 16S rRNA amplicon concentrations (ng/μL) reported by gel quantification accurately reflected bacterial burden, we performed total 16S rRNA Q-PCR and regression analysis on these independent measurements, which demonstrated strong concordance \( (r = 0.69; p < 0.001) \). This data also confirmed that samples with sufficient amplicon for array analysis had significantly greater 16S rRNA copy number compared with those unable to be analyzed by PhyloChip \( (p = 0.002) \). Therefore, gel-quantified 16S rRNA amplicon concentrations were used as a proxy for bacterial burden to examine potential relationships between airway bacterial burden and clinical variables. Of the samples analyzed by PhyloChip, bacterial burden was significantly higher in asthmatic samples compared to controls \( (p = 0.008; \text{Figure 1}) \). Bacterial burden appeared to decline with increasing methacholine PC\(_{20}\) measurements albeit this did not reach statistical significance \( (r = -0.21; p = 0.09) \), suggesting that community composition rather than actual bacterial burden may be more related to this clinical feature.

Airway microbiota structure and clinical features of asthma

Phylogenetic analysis identified 1,941 bacterial taxa (detected in at least one subject), representing 161 bacterial families (see Table E1 in the Online Repository). As a form of validation, we compared the phylogenetic distribution of airway microbiota detected by the array with reference trees for airway bacteria reported in the Human Microbiome Project (http://www.hmpdacc.org/reference_genomes.php; accessed May 13, 2010)\(^3\)\(^7\) and observed good agreement in the types of bacteria reported by both (data not shown). Community richness across asthmatic samples was variable and ranged from 48 to 1,240 taxa. Of the five healthy subjects, three exhibited low community richness (200 to 253 taxa) while two demonstrated greater richness (844 and 1,121 taxa), suggesting that variability in airway microbiota composition was not exclusive to asthmatic patients.

We found no association between study center and variation in microbiota composition, indicating that sample collection by different operators in distinct geographic regions was not a major influence on community composition in this study. Multivariate analyses also showed no association between microbiota composition and spirometry measurements (FEV\(_1\) and FVC), sputum eosinophil or neutrophil percentage, history of exacerbations or systemic corticosteroid treatment within the previous two years. However, microbiota structure differed significantly based on asthmatic or healthy group assignment \( (p_{MRPP} = 0.028) \), and NMDS analysis demonstrated that variability in airway microbiota composition was correlated with both the degree of airway hyperresponsiveness (methacholine PC\(_{20}\) concentrations; \( r = 0.56, p < 0.001; \text{Figure 2A} \) and bacterial burden \( (r = -0.36, p = 0.015; \text{Figure 2B}) \). This suggests that the types and relative abundance of bacteria present in the airways are associated with these two variables.

Airway bacterial diversity, community composition, and bronchial hyperresponsiveness

Asthmatic subjects possessed greater airway microbiota diversity than controls \( (p = 0.012) \). Methacholine PC\(_{20}\) concentrations were inversely correlated with diversity \( (r = -0.46, p < 0.003; \text{Figure 2C}) \), suggesting a strong relationship between increased airway bacterial diversity and greater bronchial hyperresponsiveness.

To determine which of the airway microbiota were most associated with bronchial hyperresponsiveness, relationships between PC\(_{20}\) and relative abundance of all taxa detected
were analyzed. After false discovery correction, ~100 taxa demonstrated a significant (p < 0.01, q ≤ 0.015) linear relationship between increasing relative abundance and greater bronchial hyperresponsiveness (Figure 3. See also Table E2 in the Online Repository). These taxa, primarily belonging to the Proteobacteria, represented 31 bacterial families such as the Comamonadaceae, Sphingomonadaceae, Nitrosomonadaceae, Oxalobacteraceae, and Pseudomonadaceae, and included a number of potentially interesting organisms. While samples were collected in a manner to minimize oral secretion contamination (e.g. use of triple-lumen protected specimen brushes, sample collection early in the procedure preferentially from upper lobes), we recognized the potential for organisms associated with the oropharynx to reside in the lower airways. Thus we investigated whether these ~100 phylotypes have previously been identified in the oral cavity by interrogating both the extensive human oral microbiome sequence database and PUBMED, using species or genus names and the search terms “oral”, “oropharynx” “mouth”, or “tongue”. The majority (87.5%) of these phylotypes were not found referenced to the oral cavity in either database.

Post-treatment bronchoscopy was not performed in the parent study, therefore examination of relationships between clarithromycin-induced changes in airway microbiota composition and treatment outcomes was not possible. Instead, we explored whether relationships existed between pre-treatment airway microbiota and post-treatment clinical outcomes. Airway bacterial diversity was significantly greater in asthmatic subjects who demonstrated a significant reduction in bronchial reactivity in response to clarithromycin treatment (defined as at least a doubling in methacholine PC_{20} dose before and after treatment) than those with a smaller reduction (p = 0.03, Figure 4). This pattern was not evident in patients who received placebo intervention (p = 0.64).

16S rRNA clone library-sequencing validation

The PhyloChip, like other array technologies, is potentially susceptible to cross-hybridization, despite features of the array design intended to minimize this. To validate array-based findings, 16S rRNA clone libraries were constructed utilizing the same amplicon pool hybridized to the array for a subset of asthmatics (n=6). An average of 333 clones per sample were analyzed yielding ~2,000 non-chimeric sequences representing 197 taxa, the majority of which were present in more than one sample. The majority of bacterial sub-families identified by sequencing were also present in the corresponding array dataset, validating large numbers of array-based positive hits (Table II). Two sub-families (Fusobacteriaceae sub-family 3 and Prevotellaceae sub-family 1) were detected by sequencing only in two samples (one sub-family per sample). However, taxa within these sub-families were very close (pf =0.84 and 0.75, respectively) to the conservative arbitrary cut-off (pf ≥ 0.9) used to define array-based presence or absence. Clone library analysis also indicated that two samples (AB01 and AB04) were each dominated by a specific bacterial family. The majority of sequences for sample AB01 were classified as Pasteurellaceae sub-family 1 [this sample also exhibited the lowest array-reported community richness (48 taxa)], while for subject AB04, a majority of the sequences belonged to the Cellulomodaceae.

Discussion

To our knowledge this is the first study to examine whether specific aspects of the airway microbiota are related to relevant clinical or physiologic features of asthma. Our findings suggest that bacterial diversity, variations in community composition, and the relative abundance of specific phylotypes, are associated with the degree of bronchial hyperresponsiveness in asthmatics administered inhaled corticosteroids. This culture-independent study also expands the repertoire of organisms of potential relevance to asthma pathogenesis within this cohort beyond those previously implicated. These data, together
with recent studies in other asthmatic patients\textsuperscript{7}, suggest that the microbiome of the airways, as with other discrete host niches, may be an important contributor to asthma pathophysiology and to the heterogeneity of disease\textsuperscript{39–41}.

Overall, these findings highlight two key aspects in defining airway health. The existence of a bronchial microbiota suggests that the bronchial tree is unlikely to be completely sterile, and that the composition of its microbiome may directly or indirectly modulate airway function. The role of airway microbiota in airway disease is under-investigated compared with other human niches such as the intestinal microbiota, which has a demonstrated role in chronic inflammatory gastrointestinal diseases\textsuperscript{10, 42}. That a bronchial epithelia-associated microbiota exists is not entirely unexpected, particularly in the setting of airway disease and/or inhaled corticosteroid (ICS) therapy. Specific bacterial communities persist in the airways despite antimicrobial treatment in patients with COPD and cystic fibrosis\textsuperscript{8, 19}. Recently, Hilty and colleagues also described airway microbiota in patients with mild to moderate asthma (all on ICS therapy), COPD, and healthy subjects\textsuperscript{7}. Those with airway disease had an increased prevalence of pathogenic Proteobacteria compared to controls, although relationships to clinical measurements were not examined in their study.

Complex microbial communities are now recognized to reside in various host mucosal sites\textsuperscript{43}, where perturbations of community structure are associated with disease\textsuperscript{6, 8, 10, 42}. Moreover, the airway epithelium is increasingly recognized as important in immunologic responses to environmental and microbial exposures\textsuperscript{44}. Thus, the airway microbiome could potentially influence presentations of asthma. As such, our findings of specific community relationships with bronchial hyperresponsiveness, in the context of a well-characterized asthmatic cohort receiving standardized baseline treatment, implicate additional bacterial groups in asthma. These include a \textit{Nitrosomonas} spp. possessing a functional nitric oxide reductase\textsuperscript{45}. Their relative abundance may be a microbial indicator of airway concentrations of nitric oxide which, though not assessed in this study, reflect airway inflammation\textsuperscript{46} and correlate with measures of airway hyperresponsiveness\textsuperscript{47}. Also notable is \textit{Oxalobacter formigenes} (Oxalobacteraceae), an anaerobic bacterium known to be susceptible to macrolide antibiotics\textsuperscript{48}. Other bacteria of interest include the Comamonadaceae and Sphingomonadaceae. Members of the Comamonadaceae, previously identified in cystic fibrosis patients\textsuperscript{49}, possess steroid-responsive degradation pathways\textsuperscript{50, 51}, and their presence could plausibly be related to the selective pressure of ICS therapy. This raises the intriguing possibility that steroid non-responsiveness, as observed in some asthmatics despite adherence to therapy\textsuperscript{52}, may be due in part to the presence of airway bacteria with steroid-degrading capacity.

Given the existence of airway microbiota, it is conceivable that coincident presence of multiple potentially pathogenic bacteria contribute to persistent airway inflammation in asthma. This possibility is supported by the finding of neutrophilia in bronchial airway samples of some patients with severe asthma despite regular use of high-dose inhaled corticosteroids\textsuperscript{53}. Such inflammation could represent an appropriate response by the host to inappropriate airway microbial colonization. For example, Sphingomonadaceae, members of which were significantly correlated with bronchial hyperresponsiveness, are characterized by the presence of cell membrane glycosphingolipids, which are recognized by and can activate invariant natural killer T (iNKT) cells resulting in induction of IL-4 and IL-13\textsuperscript{54, 55}. Glycolipid activation of iNKT cells can also induce airway hyperreactivity independent of conventional CD4\textsuperscript{+} T cells\textsuperscript{56}. While the presence of iNKT cells in asthmatic airways remains controversial\textsuperscript{57, 58}, our findings suggest a possible role for Sphingomonadaceae in airway pathophysiology and the possibility that the observed variability of iNKT cell populations in asthmatics may depend in part on the relative abundance of these species.
We recognize that this pilot study is limited by the relatively small number of subjects, especially of healthy controls, and by the absence of asthmatics not taking an ICS. Nonetheless, the data indicate that airway bacterial burden in individuals without airway disease is much lower than in asthma patients requiring ICS therapy. As all asthmatics were required to be on standardized ICS for the parent study, we are unable to infer whether the higher bacterial burden among asthmatics is a function of the disease itself or ICS treatment, although the relationships of bacterial burden, and the composition and diversity of the microbiota, to bronchial hyperresponsiveness, are robust. Determining the effects of ICS use on the airway microbiome will require further investigation and is an important question, given the wide use of ICS therapies in airway diseases, which, among patients with chronic obstructive pulmonary disease, has been associated with an increased risk of pneumonia.

The array-based analysis in this study identified a greater diversity of airway microbiota than has previously been described, although to our knowledge there has been only one other study involving asthmatics that applied 16S rRNA phylogenetic analysis. In addition to clone library-sequencing validations, our results also agreed with those of Hilty et al., in that we detected all bacterial phyla and genera identified in their study of the lower airways of eleven adult and thirteen pediatric asthma patients on ICS therapy. The PhyloChip platform permits in-depth analysis of relatively large numbers of samples using a standardized assay that applies several levels of stringent criteria for determining the microbiota profile in a given sample. It is impractical to perform sequence corroboration for every array-based positive hit. Moreover, the extent of microbial diversity detected is dependent on the community structure and the depth of sequencing performed. Our study included the largest number of clones sequenced to date for asthmatic airway samples and confirmed the presence of many array-detected phylotypes, indicating that complex microbiota do exist in this niche.

Finally, we observed that asthmatics exhibiting a significant decrease in bronchial hyperresponsiveness post-clarithromycin treatment in the parent study, possessed greater pre-treatment airway bacterial diversity than did non-responders. Despite the small number of subjects in this exploratory analysis, the trend is interesting given that prior studies have also observed decreased bronchial responsiveness following prolonged macrolide therapy.

Furthermore, while the antimicrobial susceptibility of the entire airway microbiota is unknown, conceivably a multitude of community members may be sensitive to macrolides, resulting in a reduction of bacterial diversity and/or burden upon treatment and manifesting clinically in a reduction in bronchial responsiveness. Thus, while the efficacy of macrolides in airway disease is often attributed to their anti-inflammatory properties, their effectiveness could also reflect an extended spectrum of antibacterial activity against members of the airway microbiota, whose composition, as in the setting of ICS, may influence outcomes.

In summary, several features of the airway microbial community are significantly associated with the degree of bronchial hyperresponsiveness among patients with sub-optimally controlled asthma. Despite limitations, these study findings are notable in providing the first evidence for the potential functional, physiologic and clinical relevance of the airway microbiome in asthma. Our results suggest that variations in airway microbiome structure and function may exert distinct effects that contribute to asthma heterogeneity and provide novel targets and hypotheses for future studies on disease mechanisms.

Clinical implications

Relationships between airway microbiota and clinical features of asthma were investigated using culture-independent approaches. Among sub-optimally controlled
adult asthmatics, bronchial hyperresponsiveness correlated strongly with airway microbiota composition and diversity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

16S rRNA 16S ribosomal RNA
ACRN Asthma Clinical Research Network
ACQ Asthma Control Questionnaire
FEV\textsubscript{1} forced expiratory volume in 1 second
FVC forced vital capacity
ICS inhaled corticosteroids
IgE immunoglobulin E
MRPP Multi-response Permutation Procedure and Indicator Species Analysis
NHLBI National Heart, Lung and Blood Institute
NMDS non-metric multidimensional scaling
PC\textsubscript{20} methacholine concentration inducing a 20% decrease in FEV\textsubscript{1}

References


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Figure 1. 16S rRNA bacterial burden in bronchial brushings from asthmatic and healthy subjects
Of subjects analyzed by PhyloChip (total n = 47), asthmatic subjects exhibited greater bacterial burden (measured concentration of 16S rRNA PCR product) than healthy subjects (p = 0.008, Welch’s t-test with log-transformed data). Median and interquartile ranges are noted.
Figure 2. Relationships between variability in airway microbiota composition and community diversity with bronchial hyperresponsiveness

Each circle represents the microbiota present in a single sample (n=44). 2A, 2B. NMDS ordination, based on Sorensen dissimilarity matrices, demonstrates that variability in community composition is most strongly related to bronchial hyperresponsiveness and bacterial burden. Axes have no inherent units and indicate spatial relationships of samples based on their phylogenetic (dis)similarity. Three asthmatic samples were excluded due to extreme mean relative Sorensen distances from the pooled mean. 2C. Increased diversity (higher Shannon indices) is correlated with greater bronchial hyperresponsiveness. (Pearson correlations with log-transformed data.)
Figure 3. Phylogenetic tree based on 16S rRNA representative gene sequences (GenBank accession numbers) of the ~100 bacterial taxa highly correlated with greater bronchial hyperresponsiveness (p < 0.01, q ≤ 0.015). Colors represent different bacterial families. Asterisks denote taxa with member species previously associated with clinical disease or possessing notable functional features (See also Table E2 in the Online Repository).
Figure 4. Baseline airway microbiota diversity and change in bronchial hyperresponsiveness with clarithromycin treatment

Sixteen asthmatic subjects with pre-treatment samples analyzed by microarray received clarithromycin therapy. Subjects with significant improvement in bronchial hyperresponsiveness post-clarithromycin (n=9) possessed higher pre-treatment airway bacterial diversity than those with no significant response (n=3). Four subjects with incomplete data due to study discontinuation were not included.
Table I

Baseline characteristics of subjects

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<th>Asthmatic (n=65)</th>
<th>Healthy (n=10)</th>
<th>p-value</th>
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<tr>
<td>Age (years)</td>
<td>39.3 ± 11.2</td>
<td>35.5 ± 12.9</td>
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<td>Gender (% Female:Male)</td>
<td>52:48</td>
<td>60:40</td>
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<td>FEV₁ (% predicted)</td>
<td>74.9 ± 15.6</td>
<td>94.1 ± 12.2</td>
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<td>PC₂₀ (mg ml⁻¹)</td>
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<td>Sputum eosinophil (%)</td>
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<td>Sputum neutrophil (%)</td>
<td>33.6 ± 23.7</td>
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<tr>
<td>ACQ Score</td>
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<td>Total IgE (IU ml⁻¹)</td>
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Asthmatic group

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<td>Gender (% Female:Male)</td>
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<td>FEV₁ (% predicted)</td>
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<td>PC₂₀ (mg ml⁻¹)</td>
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<td>Sputum eosinophil (%)</td>
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</tr>
<tr>
<td>Sputum neutrophil (%)</td>
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<td>ACQ Score</td>
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<td>Positive allergen skin test result (reaction to at least one allergen)</td>
<td>79% (33/42)</td>
<td>74% (17/23)</td>
<td></td>
</tr>
<tr>
<td>Total IgE (IU ml⁻¹)</td>
<td>114.6 (0.03)</td>
<td>158.3 (0.03)</td>
<td>0.4</td>
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</table>

History of last oral corticosteroid use (# subjects)

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<tr>
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<th>None since age 12</th>
<th>Age 12 – up to two years prior to study enrollment</th>
<th>Within last two years prior to study enrollment</th>
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<tr>
<td></td>
<td>15</td>
<td>13</td>
<td>14</td>
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</table>

Exacerbation events during study (# subjects)

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<th></th>
<th>Within 1 week of bronchoscopy</th>
<th>Greater than 1 week after bronchoscopy</th>
<th>No exacerbations during study</th>
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<tr>
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<td>5</td>
<td>3</td>
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</table>

Values are mean ± SD, except where noted;

^Geometric mean (CV) reported.

†Fisher’s exact p-value. NS, not significant.
* Samples with a negative 16S rRNA PCR screen or < 100 ng of total 16S rRNA PCR product for hybridization were not analyzed by PhyloChip.
Table II
Bacterial families identified by both 16S rRNA clone library-sequencing and 16S rRNA PhyloChip
Phyotypes reported at sub-family level classified by Greengenes G2 chip taxonomy (number of clones sequenced per library indicated in parentheses). Highlighted sub-families were undetected by array based on conservative criteria for determining taxon presence.

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<thead>
<tr>
<th>AB01 (374)</th>
<th>Family</th>
<th>SF</th>
<th>Phylum</th>
<th>Family</th>
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<th>Phylum</th>
<th>Family</th>
<th>SF</th>
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</thead>
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<td>Peptococc/Acidaminococc</td>
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*a* SF, sub-family