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Altered Fecal Microbiota Composition Associated with Food Allergy in Infants

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Increasing evidence suggests that perturbations in the intestinal microbiota composition of infants are implicated in the pathogenesis of food allergy (FA), while the actual structure and composition of the intestinal microbiota in human beings with FA remain unclear. Microbial diversity and composition were analyzed with parallel barcoded 454 pyrosequencing targeting the 16S rRNA gene hypervariable V1-V3 regions in the feces of 34 infants with FA (17 IgE mediated and 17 non-IgE mediated) and 45 healthy controls. Here, we showed that several key FA-associated bacterial phylotypes, but not the overall microbiota diversity, significantly changed in infancy fecal microbiota with FA and were associated with the development of FA. The proportion of abundant *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* phyla were significantly reduced, while the *Firmicutes* phylum was highly enriched in the FA group ($P < 0.05$). Abundant *Clostridiaceae* 1 organisms were prevalent in infants with FA at the family level ($P = 0.016$). FA-enriched phylotypes negatively correlated with interleukin-10, for example, the genera *Enterococcus* and *Staphylococcus*. Despite profound interindividual variability, levels of 20 predominant genera were significantly different between the FA and healthy control groups ($P < 0.05$). Infants with IgE-mediated FA had increased levels of *Clostridium sensu stricto* and *Anaerobacter* and decreased levels of *Bacteroides* and *Clostridium* XVIII ($P < 0.05$). A positive correlation was observed between *Clostridium sensu stricto* and serum-specific IgE ($R = 0.655$, $P < 0.001$). The specific microbiota signature could distinguish infants with IgE-mediated FA from non-IgE-mediated ones. Detailed microbiota analysis of a well-characterized cohort of infants with FA showed that dysbiosis of fecal microbiota with several FA-associated key phylotypes may play a pathogenic role in FA.

Food allergy (FA) is a common allergic disorder characterized by adverse immune and hypersensitivity reactions to food proteins (1). The prevalence of FA has been increasing worldwide in recent decades, reaching 6% among children <3 years of age and approximately 4% of adults in the United States, resulting in a considerable public health and economic burden (2). The key feature of FA is a T-helper type 2 (Th2)-predominant allergen-specific immune response, with the production of IgE antibodies specific for the food allergen (3). Except for IgE-mediated FA, many non-IgE reactions, which are poorly defined clinically and scientifically, are believed to be T-cell mediated. However, the etiology of FA remains unclear; a failure to establish or a breakdown in the maintenance of oral tolerance may be responsible. Generally, oral tolerance can be profoundly influenced by genetic predisposition, the route of allergic sensitization (placental, skin, and breast milk), timing and dose of allergen exposure, environment (diet, pollutants, gastric pH, and microbiota), and tissue milieu, in particular a bias toward Th2- versus tolerogenic Th1-mediated responses. No single unifying cause has been identified, but there is recent evidence showing that lack of microbial exposure during infancy is one factor responsible for FA (4–7). Clinical epidemiologic studies have postulated that a reduced microbial pressure in westernized countries underlies the increase in development of allergies. Indeed, alterations in the early gut microbiota may precede the development of allergies (7–11). Experimental evidence also demonstrates the importance of microbiota in shaping the development of the immune system (12), and disease-associated microbiota may play a pathogenic role in FA (5). Stimulation of the immune system by the commensal gut microbiota in the early stage of childhood might prevent the development of FA. Establishment of oral tolerance to dietary antigens is, at least in part, dependent on the presence of commensal microbes (13). However, a detailed assessment of the human gut microbiota for FA in infants has not been performed. Moreover, a good definition of gut microbiota and an understanding of the relationship to FA are essential in preventing and combating FA.

To date, the gut microbiota can be viewed as a forgotten "organ," exquisitely tuned to our physiology, which performs functions that we have not had to evolve on our own. A growing body of evidence suggests that gut microbiota plays an essential role in host health by processing energy from food, protecting intestinal epithelial cells from injury, and promoting local and systemic immunity (12, 14), which has been extensively studied in

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TABLE 1 Descriptive data of infants in the study

Parameter	Value for infant group:		
	Healthy control (n = 45)	IgE mediated (n = 17)	Non-IgE mediated (n = 17)
Age of infants (mo; means ± SD) (range)	5.60 ± 2.33 (2–11)	5.12 ± 1.83 (2–8)	5.00 ± 1.37 (3–8)
Weight at birth (g; means ± SD)	3,671 ± 605	3,536 ± 584	3,579 ± 608
Proportion of males, no. (%)	21 (46.7)	9 (52.9)	9 (52.9)
Vaginal delivery, no. (%)	42 (93.3)	15 (88.2)	14 (82.3)
Breast-fed, no. (%)	38 (84.4)	16 (94.1)	15 (88.2)
Vomiting, no. (%)	0 (0)	16 (94.1)	8 (47.1)
Diarrhea (no. of times; means ± SD)	2.09 ± 0.73	5.82 ± 2.16	5.53 ± 1.87
Stool characteristics, no.			
Normal	45	0	0
Watery	0	1	5
Mucous	0	4	1
Bloody	0	7	7
Mucous bloody	0	5	4
Skin prick test (>6 mm; no. positive)			
Cow's milk	ND[a]	4	4
Egg	ND	4	6
Wheat	ND	1	4
Nuts	ND	3	2
Peanut	ND	1	1
Fish	ND	1	1
Shrimp	ND	0	1
Soy bean	ND	0	2
Specific-IgE of >0.35 KU/liter (no. of patients)[b]			
Cow's milk	0	12	0
Egg	0	11	0
Wheat	0	1	0
Nuts	0	0	0
Peanut	0	3	0
Fish	0	0	0
Shrimp	0	2	0
Soy bean	0	0	0
IL-10 (pg/ml; means ± SD)	313.4 ± 58.3	209.5 ± 62.0	189.0 ± 62.0
Diagnostic food challenge (positive/negative)	0/45	17/0	17/0
Eczema, no.	0	10	10

[a] ND, not detected.
[b] KU, kilo-international units.

recent years using culture-independent molecular methods. The next-generation DNA sequencing technologies, including high-throughput 454 pyrosequencing, provide a large number of sequencing reads in a single run, resulting in a large sampling depth and the detection of low-abundance taxa. Thus, the results of studies using high-throughput sequencing technologies have revolutionized our understanding of the gut microbiota in healthy and disease conditions.

The aim of this study was to identify differences in gut microbiota between healthy and FA subjects in Chinese infants using massively parallel barcoded 454 pyrosequencing targeting the 16S rRNA gene V1-V3 hypervariable regions to verify our hypothesis that altered human gut microbiota was associated with the development of FA in infants. Using feces as a proxy for gut microbiota, we sought to identify specific microbiota signatures for FA and their relationships to clinical patterns and physiologic measures.

MATERIALS AND METHODS

Recruitment of subjects. Seventy-nine infants were enrolled (34 infants with FA and 45 healthy controls) (Table 1). FA was diagnosed and assessed according to the National Institute for Health and Clinical Excellence (NICE) guidelines (1, 15). Infants with suspected FA who were brought to the Department of Pediatrics of Peking University Third Hospital for evaluation between June 2012 and June 2013 had their FA established by means of clinical history or symptoms and confirmed with skin prick tests (SPTs) and oral food challenges with successive doses of milk, eggs, wheat, nut, peanuts, fish, shrimp, and soy beans. Measurement of serum-specific IgE (sIgE) to the different food allergens mentioned above (FEIA; Pharmacia Diagnostics, Uppsala, Sweden) was performed on all subjects according to the protocol of the manufacturer. Only infants with suspected FA undergoing all three tests required for this study (oral food challenge, determination of sIgE, and SPTs) were included in the study. Control infants were from the same cohorts and did not have allergic manifestations or increased total or specific IgE levels. Serum was also analyzed for

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interleukin-10 (IL-10) using the human IL-10 immunoassay kit (eBioscience, San Diego, CA, USA). The following exclusion criteria were established: allergic rhinitis; atopic eczema; asthma; use of antibiotics, probiotics, prebiotics, or synbiotics in the previous month; and known active bacterial, fungal, and/or viral infection(s). The study protocol was approved by the Ethics Committee of Peking University Third Hospital (Beijing, China). Informed written consent was obtained from the parents or guardians of all participants prior to enrollment.

Sample collection and DNA extraction. Approximately 2 g of a fresh fecal sample was collected in a sterile plastic cup when the infant underwent initial examination at the hospital and was kept in an ice box. Samples for bacterial genomic DNA extraction were transferred immediately to the laboratory and stored at −80°C after preparation within 15 min until use. DNA was extracted from 300 mg of feces using a QIAamp DNA stool minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, with additional glass bead-beating steps on a mini-bead-beater (FastPrep; Thermo Electron Corporation, Boston, MA, USA). The amount of DNA was determined using a NanoDrop ND-1000 spectrophotometer (Thermo Electron Corporation); the integrity and size were checked by 1.0% agarose gel electrophoresis containing 0.5 mg/ml ethidium bromide. All DNA was stored at −20°C before further analysis.

PCR and pyrosequencing. The bacterial genomic DNA was amplified with the 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 533R (5′-TTACCGCGGCTGCTGGCAC-3′) primers specific for the V1-V3 hypervariable regions of the 16S rRNA gene (16). Each forward primer incorporated FLX Titanium adapters and a sample barcode at the 5′ end of the reverse primer to allow all samples to be included in the single 454 FLX sequencing run (see Table S1 in the supplemental material). All PCRs were performed in 50-μl triplicates and combined after PCR. The products were extracted with the QIAquick gel extraction kit (Qiagen) and quantified on a NanoDrop ND-1000 spectrophotometer, QuantiFluor-ST fluorometer (Promega, USA), and Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Equimolar concentrations of 79 samples were pooled and sequenced on a 454 Life Sciences genome sequencer FLX system (Roche, Basel, Switzerland) according to the manufacturer's recommendations.

Bioinformatics and statistical analysis. Raw pyrosequencing reads obtained from the sequencer were denoised using Titanium PyroNoise software (17–19). The resulting pyrosequencing reads were filtered according to barcode and primer sequences using a combination of tools from Mothur (version 1.25.0; http://www.mothur.org) and custom Perl scripts. Preliminary quality control steps included the removal of sequences shorter than 150 nucleotides with homopolymers longer than 8 nucleotides, those with an average quality score of <25, and all reads containing ambiguous base calls or >2 incorrect primer sequences. Using the Mothur implementation of the ChimeraSlayer algorithm (20), chimera sequences arising from the PCR amplification were detected and excluded from the denoised sequences. The high-quality sequences were assigned to samples according to barcodes. The high-quality reads were clustered into operational taxonomic units (OTUs) using Mothur (21). The OTUs that reached a 97% nucleotide similarity level were used for alpha diversity (Shannon, Simpson, and evenness indices), richness (ACE and Chao1), Good's coverage, Venn diagram, and rarefaction curve analysis using Mothur. A heatmap was generated on the basis of the relative abundance of OTUs using R (version 2.15; The R Project for Statistical Computing; http://www.R-project.org). Phylogenetic beta diversity measures, such as unweighted UniFrac distance metrics analysis, was performed using OTUs for each sample using the Mothur program. Principal coordinate analysis (PCoA) was conducted according to the distance matrices created by Mothur, and three-dimensional graphical outputs were drawn using SigmaPlot (version 12.0; Systat Software Inc., USA) (22).

Taxonomy-based analyses were performed by classifying each sequence using the Naive Bayesian Classifier program of the Michigan State University Center for Microbial Ecology Ribosomal Database Project (RDP) database (http://rdp.cme.msu.edu/) with a 50% bootstrap score (23). The Metastats program from Mothur was used to identify statistically different phylotypes among groups (21). Microbiome features of healthy controls were compared to those of patients with FA with Metastats using the P value and the false discovery rate (Q value) for nonnormal distributions. Only taxa with average abundances of >1%, $P < 0.05$, and low Q values (i.e., low risk of false discovery) were considered significant (24). The characterization of microorganismal features differentiating the fecal microbiota specific to FA was performed using the linear discriminant analysis (LDA) effect size (LEfSe) method (http://huttenhower.sph.harvard.edu/lefse/) for biomarker discovery, which emphasizes both statistical significance and biological relevance (25). With a normalized relative abundance matrix, LEfSe uses the Kruskal-Wallis rank sum test to detect features with significantly different abundances between assigned taxa and performs LDA to estimate the effect size of each feature. A significance alpha of 0.05 and an effect size threshold of 2 were used for all biomarkers discussed in this study.

The correlation between variables was computed using the Spearman rank correlation. Statistical analyses were performed using the SPSS data analysis program (version 16.0; SPSS Inc., Chicago, IL, USA). All tests for significance were two sided, and $P < 0.05$ was considered statistically significant.

Sequence read accession number. The sequence data from this study have been deposited in the GenBank Sequence Read Archive under accession number SRP028835.

RESULTS

Overall structural changes of FA-associated fecal microbiota. Of 770,480 high-quality sequences produced from 79 samples, accounting for 61.5% of valid sequences (1,251,846 reads in total) according to barcode and primer sequence filtering, an average of 9,752 (range, 4,031 to 15,596) sequences per barcoded sample was recovered for downstream analysis. Thus, a total of 433,554 sequences were obtained from healthy infants for phylogenetic analysis, while 336,926 sequences were obtained from infants with FA. The total number of unique sequences from the 2 groups was 11,501 and represented all phylotypes. Specifically, 7,268 species-level OTUs in healthy control infants and 6,495 OTUs in infants with FA were delineated at 97% similarity level. The summary information is shown in Table 2, and detailed characteristics of each sample are shown in Table S2 in the supplemental material. Good coverage was nearly 99.0% for all sequences in the 2 groups, indicating a greater sequencing depth for FA-associated fecal microbiota investigation in infants. Analysis of alpha diversity, such as Shannon and Simpson indices, showed that the infants with FA, as a whole, have average phylogenetic diversity of fecal microbiota similar to that of healthy controls (see Fig. S1). Although the Shannon index was lower in infants with FA and the Simpson index was higher, these differences failed to reach statistical significance ($P >$ 0.05). By rarefaction analysis estimates, the trend of species richness in infants with FA was also similar to that of healthy controls (see Fig. S2A and S3). Based on the observed OTU analysis, a long tail in the rank abundance curves was observed, indicating that the majority of OTUs were present at low abundance (see Fig. S2B). Beta diversity analysis indicates the extent of similarity between microbial communities by measuring the degree to which membership or structure is shared between communities. Due to significant interindividual variations, the fecal microbiota from two groups could not be divided into different clusters according to community composition using unweighted UniFrac metrics (see Fig. S4) and could not be separated clearly by principal coordinates analysis (see Fig. S5); however, the clustering was complemented by an analysis of bacterial

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TABLE 2 Comparison of phylotype coverage and diversity estimation of the 16S rRNA gene libraries at 97% similarity from the pyrosequencing analysis

Group	No. of reads	No. of OTUs[a]	Good's[b] (%)	Richness estimator				Diversity index		
				ACE	95% CI	Chao1	95% CI	Shannon	Simpson	Evenness[c]
Healthy control	433,554	7,268	99.09	21,913	21,323–22,527	14,853	14,201–15,567	5.003	0.0178	0.0077
Food allergy	336,926	6,495	98.89	21,671	21,044–22,325	14,531	13,802–15,332	4.775	0.0235	0.0066

[a] The operational taxonomic units (OTUs) were defined with 97% similarity level.
[b] The coverage percentage (Good's) and richness estimators (ACE and Chao1) were calculated using Good's method, and diversity indices (Shannon and Simpson) were calculated using the Mothur program. CI, confidence interval.
[c] The Shannon index of evenness was calculated with the formula $E = H/\ln(S)$, where E is evenness, H is the Shannon diversity index, and S is the total number of sequences in that group.

richness using the number of shared and unique OTUs in the two groups by a Venn diagram, which was generated to compare OTUs between two groups. At the 97% similarity level, 2,262 OTUs were shared between the 2 groups, which accounted for 34.8% and 31.1% of the total OTUs in infants with and without FA, respectively (see Fig. S6). These results indicated that infants shared a core set of bacteria in fecal microbiota regardless of health status. In contrast to previous studies, which focused only on predominant bacteria (4, 6), the present study demonstrated an unaltered overall diversity of FA-associated fecal microbiota in infants but fewer OTUs and phylotypes, which could result from the greater depth of sequencing coverage.

Associations between fecal microbiota and infancy FA. A taxon-dependent analysis using the RDP classifier was conducted to describe the composition of infancy FA-associated fecal microbiota. Eleven phyla and two candidate divisions (TM7 and OD1) were found. Three phyla (*Firmicutes*, *Bacteroidetes*, and *Proteobacteria*) were the most predominant in infancy fecal samples and comprised >97% of all sequences, which showed a higher proportion of *Proteobacteria* in infants than in adults (26). In total, sequences from fecal microbiota could be classified as 270 genera, with 237 genera in healthy infants and 183 genera in infants with FA. Of the total number of genera identified in fecal microbiota, 17 predominant genera were detected in healthy infants and 18 predominant genera were detected in infants with FA, with 11 predominant genera being shared by both groups. The predominant genera were defined as having >1% of the total DNA sequences (27). These predominant genera accounted for 89.89% and 93.33% of the total sequences from healthy controls and infants with FA, respectively. The two most predominant genera were *Bacteroides* and *Veillonella* in healthy controls and *Clostridium sensu stricto* and *Bacteroides* in infants with FA. Among the shared predominant genera in both groups, *Bacteroides* was a member of the phylum *Bacteroidetes*, *Escherichia*/*Shigella* and *Klebsiella* belonged to the phylum *Proteobacteria*, and the other 8 genera were all classified as *Firmicutes*.

To investigate the associations between fecal microbiota and FA and to identify specific phylotypes responding to FA, we examined the composition of infancy fecal microbiota using Metastats. At the phylum level, the proportion of *Firmicutes* and *Fusobacteria* increased in FA cases, whereas the proportion of *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* decreased ($P < 0.05$) (see Table S3 in the supplemental material). At the family level, *Clostridiaceae* 1, *Cytophagaceae* and *Nocardiaceae* were prevalent in infants with FA, while *Alcaligenaceae*, *Clostridiales incertae sedis* XIII, *Phyllobacteriaceae*, *Xanthomonadaceae*, *Flavobacteriaceae*, and *Moraxellaceae* were enriched in healthy controls (Fig. 1A). At the genus level, 74 genera were identified differentially between the 2 groups, including 20 predominant (>1% of the total sequences in either group) and 54 less predominant genera (see Table S4). Among the predominant differential genera, 11 genera, such as *Clostridium sensu stricto*, *Enterococcus*, *Escherichia*/*Shigella*, *Lactobacillus*, *Staphylococcus*, *Faecalibacterium*, *Clostridium* XIVa, *Anaerostipes*, *Prevotella*, *Clostridium* XVIII, and *Flavonifractor*, showed increases in FA, whereas 9 genera, including *Bacteroides*, *Streptococcus*, *Veillonella*, *Klebsiella*, *Blautia*, *Clostridium* XI, *Lachnospiracea incertae sedis*, *Megamonas*, and *Megasphaera*, showed decreases ($P < 0.05$). Intriguingly, FA-enriched differential phylotypes, such as the genera *Enterococcus* and *Staphylococcus*, were negatively correlated with anti-inflammatory cytokines, such as IL-10 ($P < 0.05$) (Fig. 1B and C). Sixteen of the 20 differentially predominant genera belonged to the phylum *Firmicutes*, and 2 each of the remaining 4 belonged to *Bacteroidetes* and *Proteobacteria*. Interestingly, well-known probiotic bacteria, such as *Lactobacillus* and *Bifidobacterium* (28), were found to be significantly increased in infants with FA.

To identify bacterial taxa that had sequences that were more abundant in infants with FA than in healthy controls, a metagenomic biomarker discovery approach (LEfSe) was used to assess the size of effect of each differentially abundant taxon. Using LEfSe, we found that *Clostridium sensu stricto* and *Butyricicoccus* sequences were significantly enriched in infants with FA, as were sequences from the families *Clostridiaceae* 1 and *Ruminococcaceae* (Fig. 1D and E). Other taxa were enriched in healthy controls, but the LDA enrichment scores were lower by one order of magnitude or more. The high abundance of *Clostridium sensu stricto* sequences, with an average relative abundance of >20% of the total bacterial sequences, was a feature of some, but not all, infants with FA. Our present data showed that the aberrant compositions of fecal microbiota were associated with the development of infancy FA.

Phylotype signatures for IgE-mediated and non-IgE-mediated FA. Consistent with our comparisons of fecal microbiota between healthy controls and infants with FA, no significant differences were detected in the overall microbial diversity between IgE-mediated and non-IgE-mediated FA ($P > 0.05$). However, further analysis suggested that several key phylotypes affected the development of IgE-mediated FA. Using LEfSe, we compared the infancy FA-associated fecal microbiota between IgE-mediated and non-IgE-mediated FA to identify specific phylotypes with OTU levels. We showed that *Clostridium sensu stricto* and *Anaerobacter* sequences were significantly enriched in the IgE-mediated FA, while *Lachnospiraceae*, *Bacteroides*, *Erysipelotrichia*, *Rhodococcus*, *Nocardiaceae*, and *Clostridium* XVIII sequences were signifi-

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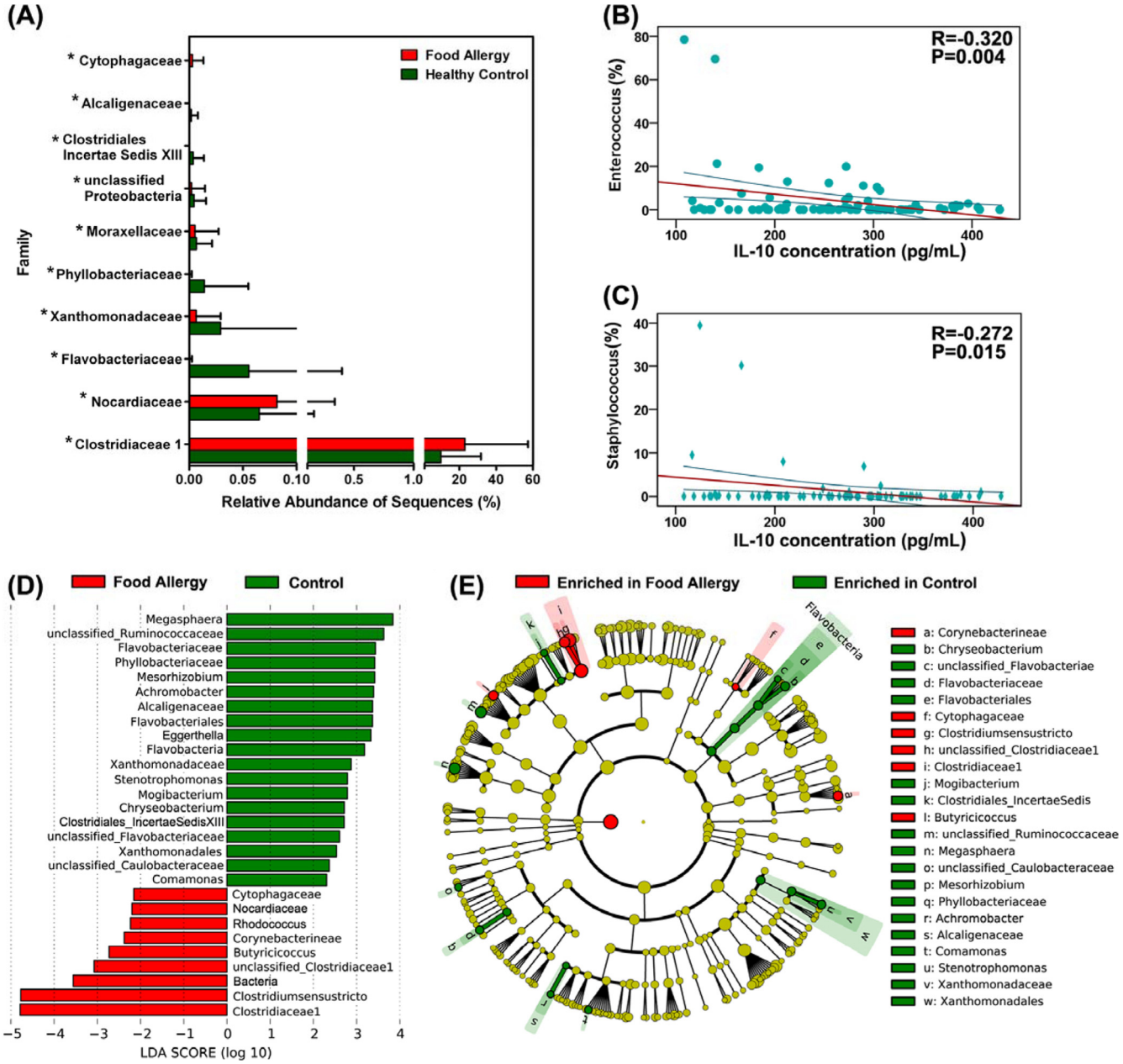

FIG 1 Comparison of relative abundance at the bacterial family level between infants with food allergy and healthy controls. (A) Asterisks indicate $P < 0.05$. Correlation between interleukin-10 and the relative abundance of the genera *Enterococcus* (B) and *Staphylococcus* (C). The Spearman rank correlation (R) and probability (P) were used to evaluate statistical importance. (D) Linear discriminant analysis (LDA) coupled with effect size measurements identifies the most differentially abundant taxons between infants with food allergy and healthy controls. Healthy control-enriched taxa are indicated with a positive LDA score (green), and taxa enriched in food allergy have a negative score (red). Only taxa meeting an LDA significant threshold of >2 are shown. (E) A cladogram representation of data shown in panel A. Red, food allergy-enriched taxa; green, taxa enriched in healthy controls. The brightness of each dot is proportional to its effect size.

cantly enriched in the non-IgE-mediated FA, which could be used as a potential biomarker for IgE-mediated FA (Fig. 2A and B). Statistical analysis identified four genera that were differentially abundant between the two groups ($P < 0.05$) (Fig. 2C). The genera of *Clostridium sensu stricto*, *Anaerobacter*, and *Clostridium* XVIII belonged to the phylum *Firmicutes*, and *Bacteroides* belonged to the phylum *Bacteroidetes*. The increased abundance of *Clostridium sensu stricto* and *Anaerobacter* and the decreased abundance of *Bacteroides* and *Clostridium* XVIII could be used to discriminate different types of FA. We then used the Kendall tau rank correlation coefficient to directly measure the correlation between sIgE and the relative abundance of the key phylotypes in fecal microbiota separately for IgE-mediated and non-IgE-mediated FA. With significant interindividual variability, we identified that only one genus, *Clostridium sensu stricto*, significantly correlated with sIgE in infants with FA ($R = 0.655$, $P < 0.001$), while the other key phylotypes showed no strong correlations with sIgE (Fig. 2D). The genus *Clostridium sensu stricto* was proportionally

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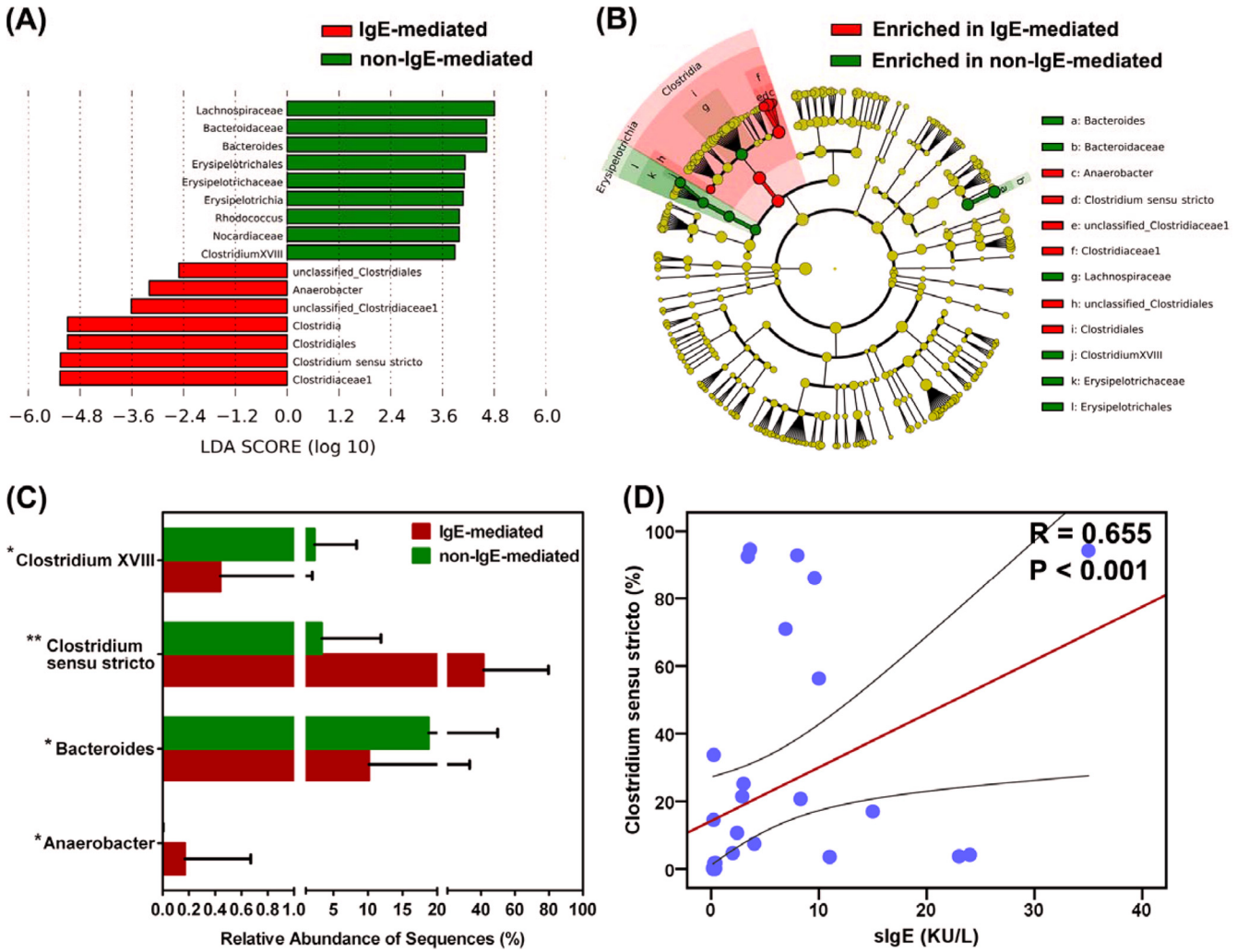

FIG 2 LEfSe identifies the most differentially abundant taxons between IgE-mediated food allergy infants and non-IgE-mediated food allergy ones. Non-IgE-mediated food allergy-enriched taxa are indicated with a positive LDA score (green), and taxa enriched in IgE-mediated food allergy have a negative score (red). (A) Only taxa meeting an LDA significant threshold of >2 are shown. (B) A cladogram representation of data shown in panel A. Red, IgE-mediated food allergy-enriched taxa; green, taxa enriched in non-IgE-mediated food allergy infants. The brightness of each dot is proportional to its effect size. (C) The relative abundance of different genera obtained in fecal microbiota from IgE-mediated and non-IgE-mediated food allergy infants. The Mann-Whitney test was used to evaluate the two groups. *, $P < 0.05$; **, $P < 0.01$. (D) Correlation between specific serum IgE and the relative abundance of *Clostridium sensu stricto*. The Spearman rank correlation (R) and probability (P) were used to evaluate statistical importance. KU/L, kilo-international units per liter.

associated with sIgE, indicating that infants with IgE-mediated FA carried a greater load of this bacterium (see Fig. S7). Interestingly, the non-IgE-mediated FA infants carried significantly less of this bacterium ($P < 0.05$).

DISCUSSION

We used barcoded multiplexed 454 pyrosequencing to examine the relationship between fecal microbiota and FA in Chinese infants. This approach allowed a relatively comprehensive description of fecal microbiota associated with FA. This study also provided an interindividual comparison of fecal microbiota to identify the key phylotypes that might be associated with the development of infancy FA. Alterations in gut microbiota have been reported in patients with allergic diseases, although it is also possible that these changes precede the development of allergic manifestations and are a cause rather than (or as well as) a consequence. Unlike previous studies, which only focused on predominant bacterial phylotypes in allergic diseases, the work presented here was designed to better understand the alterations of overall bacterial diversity and composition in infants with FA at a much deeper level (4, 6, 29). Previous studies have shown that reduced diversity of the early intestinal microbiota during infancy is associated with an increased risk of allergic disease such as FA using DNA fingerprinting techniques, which can only detect bacteria with a relative abundance of >1% (4, 6, 30). Fewer OTUs and genera were found in infants with FA; however, the current study demonstrated an unaltered overall bacterial diversity of fecal microbiota in infants with FA (5). This discrepancy might be ascribed to a different depth of coverage for fecal microbiota with different methods. Our study showed that microbial diversity in the feces of infants was much greater than that of previous estimates, which were based on conventional molecular techniques.

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Good's coverage was >99.0% for all sequences in the FA and healthy control groups, indicating that less than one additional phylotype would be expected for every 100 additional sequenced reads. This level of coverage indicated that the 16S rRNA sequences identified in these groups represent the majority of bacterial sequences present in the samples under study, which could approach to the real world of infancy fecal microbiota. With an average of nearly 10,000 reads per sample, a large number of rare taxa present at relatively low abundances were detected, which influenced the overall bacterial diversity of fecal microbiota (31). The major strength of the 454 pyrosequencing method used in the current study is allowing a much deeper and more comprehensive analysis of changes in the gut microbiota responding to FA than in previous reports.

Consistent with previous studies using molecular methods, we sampled the fecal microbiota from infants at a single time point. In contrast to those well-controlled experimental FA animal models (5), profound interindividual variations were found in the composition of the fecal microbiota of infants with FA. In fact, the establishment of infancy gut microbiota was influenced by a number of factors, including delivery and feeding modes, birth order, degree of social exposure, and environmental bacterial content, which subsequently influenced the risk of atopic manifestations (11, 32–35). The interindividual variation implied that the same phylotypes occupy somewhat different niches in different individuals and have different linkages to other taxa, displaying different responses to FA. Generally, interindividual variability was greater than differences within the same subjects over time (36). The relative long-term stability of gut microbiota within individuals could be used to monitor the microbiota of patients with FA (37). Therefore, our data at a single time point could be used to identify the associations between specific phylotypes and FA with the relatively large cohort of subjects enrolled in our study.

Although the overall bacterial diversity of the fecal microbiota was unaltered in the present study, statistical analysis showed that some bacterial phylotypes were associated with the development of FA in infants. Most of these phylotypes were classified at the genus level but not into the higher taxonomic levels, such as bacterial families and orders. Using a high-density 16S rRNA gene oligonucleotide microarray (PhyloChip), Noval et al. exhibited a specific microbiota signature characterized by coordinate changes in the abundance of taxa of several bacterial families, including the *Lachnospiraceae*, *Lactobacillaceae*, *Rikenellaceae*, and *Porphyromonadaceae*, in experimental FA mouse models (5). The present fecal microbiota analysis also revealed a dysbiosis in the feces of infants with FA. In fact, the dysbiotic microbiota alone is sufficient to drive the intestinal immune disorders (38). Recent studies in animal models, however, have reinforced the notion that commensal microbiota contribute to systemic autoimmune and allergic diseases at sites distal to the intestinal mucosa (14). In the cross-talk between intestinal microbiota and host immune homeostasis, IL-10 plays a central role in the regulation of immune responses against commensal bacteria (39). Our present study found that FA-enriched phylotypes, such as the genera *Enterococcus* and *Staphylococcus*, were negatively correlated with IL-10. In contrast to previous studies, we identified several FA-associated specific phylotypes at the genus level, not just the changing patterns of fecal microbiota (4, 6). A total of 20 differentially abundant genera were observed in infancy fecal microbiota. *Clostridium sensu stricto*, *Enterococcus*, *Escherichia/Shigella*, *Lactobacillus*, *Staphylococcus*, *Faecalibacterium*, *Clostridium* XIVa, *Anaerostipes*, *Prevotella*, *Clostridium* XVIII, and *Flavonifractor* were detected more frequently in the FA group than in the healthy control group, and the others were present more frequently in the healthy control group than in the FA group. These abundant genera could be used as potential biomarkers for diagnosing FA and selecting treatment. The other 54 less abundant genera were also differentially present in the two groups, while the roles were difficult to accurately predict. All of these features contributed to the dysbiosis of fecal microbiota in infants with FA.

The major component of infancy fecal microbiota, *Clostridium sensu stricto* (clostridial cluster I), the core genus of the family *Clostridiaceae*, was strongly associated with infancy FA. Recently, Penders et al. reported that the prevalence of *Clostridium* cluster I (*Clostridium sensu stricto*) is positively associated with atopic dermatitis at 5 and 13 weeks of age (11); however, there was still no consensus of opinion about the role of *Clostridium* cluster I (*Clostridium sensu stricto*) in allergic diseases (11, 40). The present study is the first to identify the associations between *Clostridium sensu stricto* and infancy FA. Unlike other bacteria in *Clostridium* cluster I, such as *Clostridium butyricum* (41), our data indicated that *Clostridium sensu stricto* could not suppress immune disorders but directly affect the pathogenesis of infancy FA as the most predominant bacteria. One intriguing observation was an increased abundance of *Clostridium* cluster XIVa, *Clostridium* cluster XVIII, *Lactobacillus*, *Faecalibacterium*, and *Bifidobacterium* in FA, which behaved as potent inducers of regulatory T cell (Treg) or anti-inflammatory commensal bacteria (42–45). Atarashi et al. demonstrated that a rationally selected mixture of *Clostridia* strains, such as clusters IV, XIVa, and XVIII from the human microbiota, rather than a single *Clostridia* strain can induce the production of Treg cells for immune tolerance (45). The present study seems to contradict recent studies involving the roles of the bacteria mentioned above; however, the immunomodulatory capacities of these bacteria were strain or species specific (46). Some strains in the same genus have a proinflammatory effect, whereas others are more anti-inflammatory. A previous study also demonstrated that the presence of *Bifidobacterium pseudocatenulatum*, not *B. bifidum*, in feces was associated with atopic eczema (29). This might be the reason for an increased relative abundance of well-known probiotic bacteria, such as the genera *Lactobacillus* and *Bifidobacterium*, in infancy FA (9). The three most predominant genera, *Escherichia/Shigella*, *Enterococcus*, and *Staphylococcus*, were increased significantly in infants with FA. A previous study also showed that these genera are differentially associated with eczema and respiratory problems in infants (35, 47). Another two FA-associated genera, *Anaerostipes* and *Prevotella*, were shown to be the predominant bacteria in infancy fecal microbiota. However, Abrahamsson et al. reported that there was no significant correlation between *Anaerostipes*, *Prevotella*, and atopic eczema in infants (30). The genus of *Flavonifractor*, prevailing in minimal hepatic encephalopathy with cirrhosis (48), was also shown to have an increased relative abundance in infants with FA. Our current study indicated that the prevalence of those genera mentioned above would increase the risk for infancy FA.

The present study demonstrated the relationship between infancy fecal microbiota and IgE-mediated FA for the first time. FA can be classified into IgE-mediated and non-IgE-mediated reactions. IgE-mediated FA usually manifests as immediate and acute allergic reactions within 2 h of exposure, while many non-IgE-

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mediated reactions are relatively mild and poorly defined both clinically and scientifically. In the current study, an increased relative abundance of *Clostridium sensu stricto* and *Anaerobacter* and a decreased relative abundance of *Bacteroides* and *Clostridium* XVIII might disturb immune homeostasis in infants and induce food protein sIgE production. Interestingly, the prevalence of *Clostridium sensu stricto* was positively associated with IgE-mediated FA in infants but not non-IgE-mediated FA ($P < 0.05$). The sIgE level, in combination with the relatively high abundance of *Clostridium sensu stricto*, could be used to discriminate IgE-mediated FA from non-IgE-mediated FA more specifically; however, the mechanisms for IgE induction in infants are still unknown. Identifying the mechanisms for IgE induction by *Clostridium sensu stricto* was of great value, because such an understanding could help to develop novel tools or approaches to prevent and treat infancy FA. Further studies focused on the host-microbe interactions might be helpful in unraveling the mystery of *Clostridium sensu stricto* in allergic diseases.

The present study also had several limitations that should be acknowledged. First, feces was used as a proxy for gut microbiota in the current study, which was the only realistic sample for large, noninvasive epidemiologic studies. However, fecal microbiota only represents the composition of gut microbiota in the lumen, not on the mucosal surfaces, and mucosa-associated microbiota may affected infancy FA risk primarily through direct interaction with the host. Second, several key phylotypes were detected in infancy FA; however, the exact mechanisms for FA development remain to be identified. The restoration of infancy fecal microbiota after successful treatment also should be performed to verify the role of these key phylotypes in future studies. Third, the establishment of infancy fecal microbiota with different delivery and feeding modes was not considered in the current study, as almost all infants were delivered vaginally and breastfed for >6 months, while different delivery and feeding modes would influence the composition of infancy fecal microbiota and the risk of FA. Fourth, there were no microbiome data on the fecal samples after the infants had been placed on an allergen-free diet, which would strengthen the relationships between fecal microbiota and FA. We conclude from our results that the altered infancy fecal microbiota composition was correlated with immunologic alterations and the risk of developing FA in infants. This study used a comparatively large number of well-characterized FA infants and deep-level pyrosequencing to identify key phylotypes that may associate with the pathology of FA. This is the first study to show that the high relative abundance of *Clostridium sensu stricto* is associated with an increased risk of IgE-mediated FA in infants. We have extended previous studies and provided new key bacterial phylotypes and associations; some will require further investigation to fully define their role in FA.

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We have no conflicts of interest to declare.

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Supplementary Information

Figure S1 The Shannon and Simpson indices were used to estimate diversity (i.e., a combined assessment of the number of 97% similar bacterial taxa and their abundance) of infancy fecal microbiota between infants with and without food allergy (Data shown as mean with SEM).

Figure S2 Rarefaction curves were used to estimate richness (in this case the number of taxa at a 97% similarity level) of infancy fecal microbiota between infants with and without food allergy. The vertical axis shows the number of OTUs that would be expected to be found after sampling the number of tags or sequences shown on the horizontal axis (A). Rank abundance curve of bacterial OTUs derived from two groups (B).

Figure S3 Rarefaction analysis of pyrosequencing tags in infancy fecal microbiota from infants with food allergy (n=34) and healthy controls (n=45).

Figure S4 Differentiation in bacterial communities from all infants (interindividual variations). Community differentiation was measured by using the unweighted UniFrac algorithm; the scale bar indicated the distance between clusters in UniFrac units. Red bars showed the infants with IgE-mediated food allergy, while green bars showed the infants with non-IgE-mediated food allergy.

Figure S5 3D PCoA plots of individual fecal microbiota in infants with IgE-mediated food allergy infants (n=17), non-IgE-mediated food allergy infants (n=17) and healthy controls (n=45) obtained by Mothur program and Sigmaplot. Each symbol represents a sample. Infancy fecal microbiota from infants with food allergy and healthy controls, IgE-mediated and non-IgE-mediated almost group together.

Figure S6 A Venn diagram illustrating overlap of OTUs in fecal microbiota between infants with and without food allergy.

Figure S7 Percent relative abundance for the genus *Clostridium sensu stricto* is depicted across all food allergy samples, demonstrating an IgE-mediated food allergy enrichment in

most individuals.

Table S1 List of the 79 10-bp barcodes used to tag each PCR product analyzed as part of the study.

Table S2 Comparison of phylotypes coverage and diversity estimation of the 16S rRNA gene libraries for individuals at 97% similarity from the pyrosequencing analysis.

Table S3 Differences in bacterial abundances between infants with and without food allergy in the phylum level.

Table S4 Differences in bacterial abundances between infants with and without food allergy in the genus level.

Figure S1

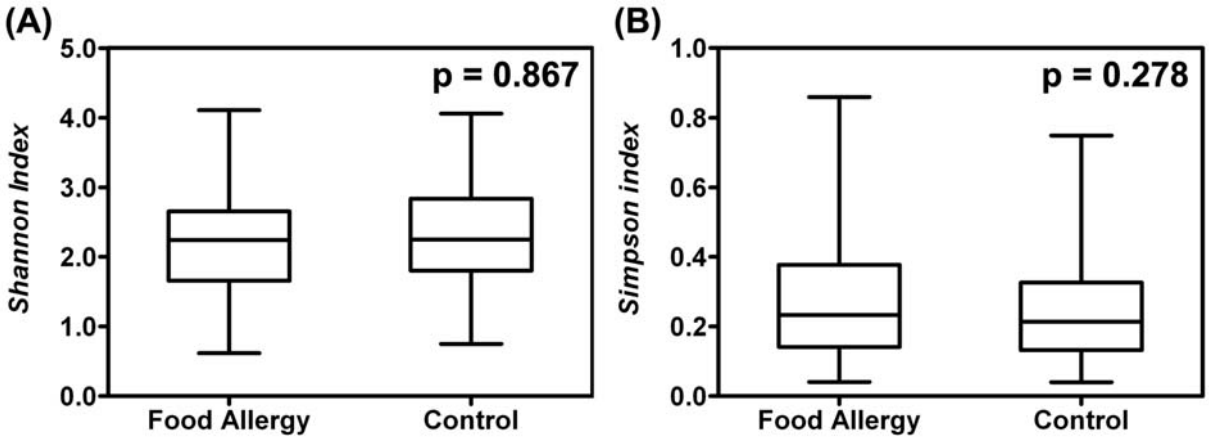
(A)
Shannon Index
5.0
4.0
3.0
2.0
1.0
0.0
p = 0.867
Food Allergy
Control
(B)
Simpson index
1.0
0.8
0.6
0.4
0.2
0.0
p = 0.278
Food Allergy
Control

Figure S2

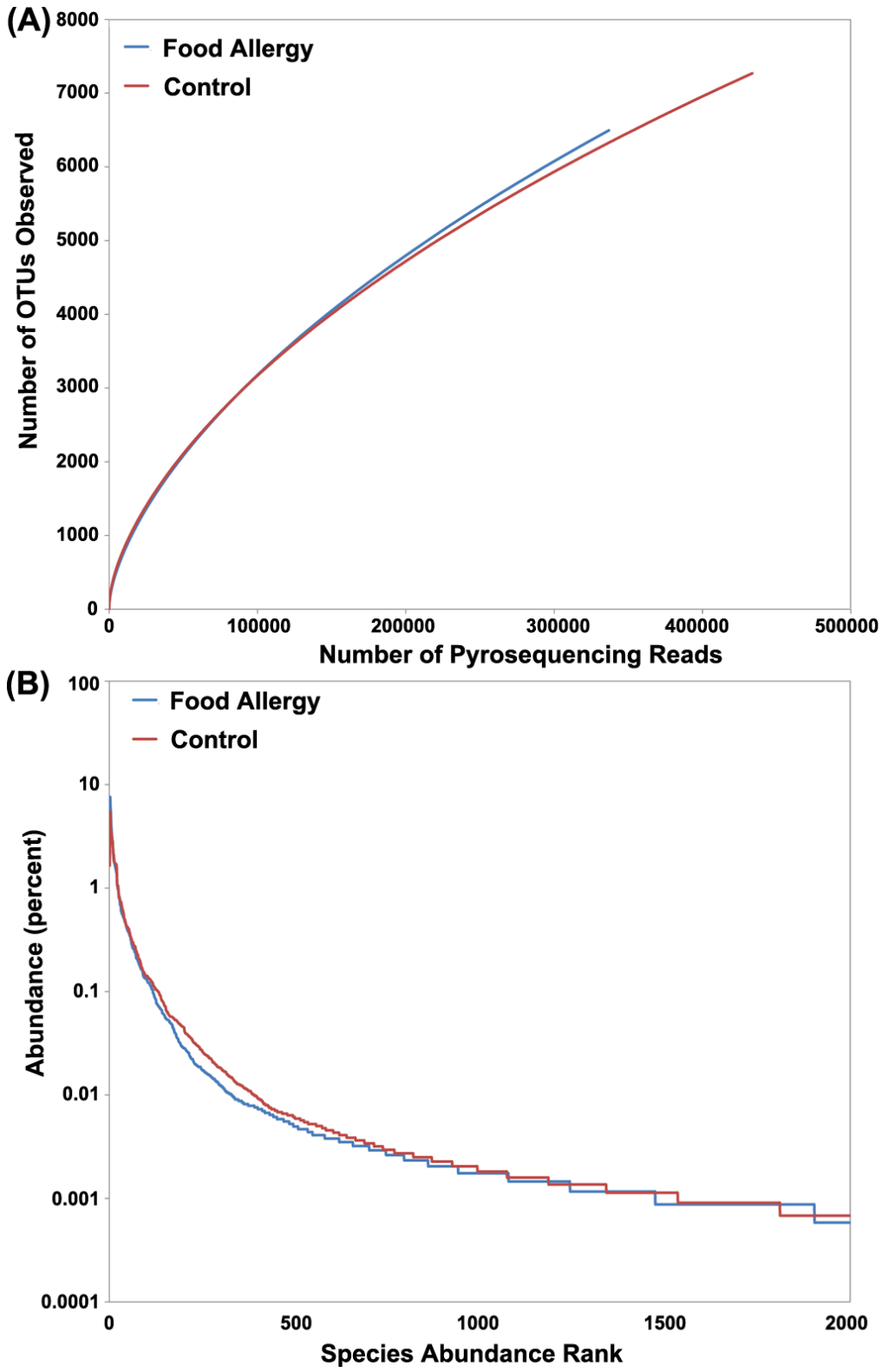
(A)
Food Allergy
Control
Number of OTUs Observed
8000
7000
6000
5000
4000
3000
2000
1000
0
0
100000
200000
300000
400000
500000
Number of Pyrosequencing Reads
(B)
Food Allergy
Control
Abundance (percent)
100
10
1
0.1
0.01
0.001
0.0001
0
500
1000
1500
2000
Species Abundance Rank

Figure S3

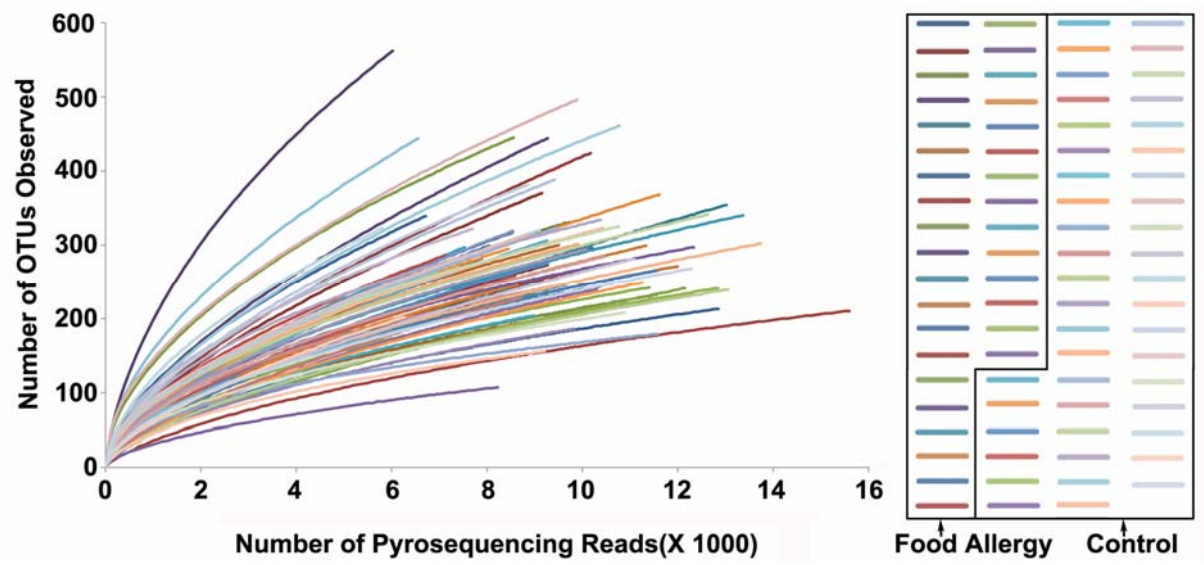
Number of OTUs Observed
600
500
400
300
200
100
0
0
2
4
6
8
10
12
14
16
Number of Pyrosequencing Reads(X 1000)
Food Allergy
Control

Figure S4

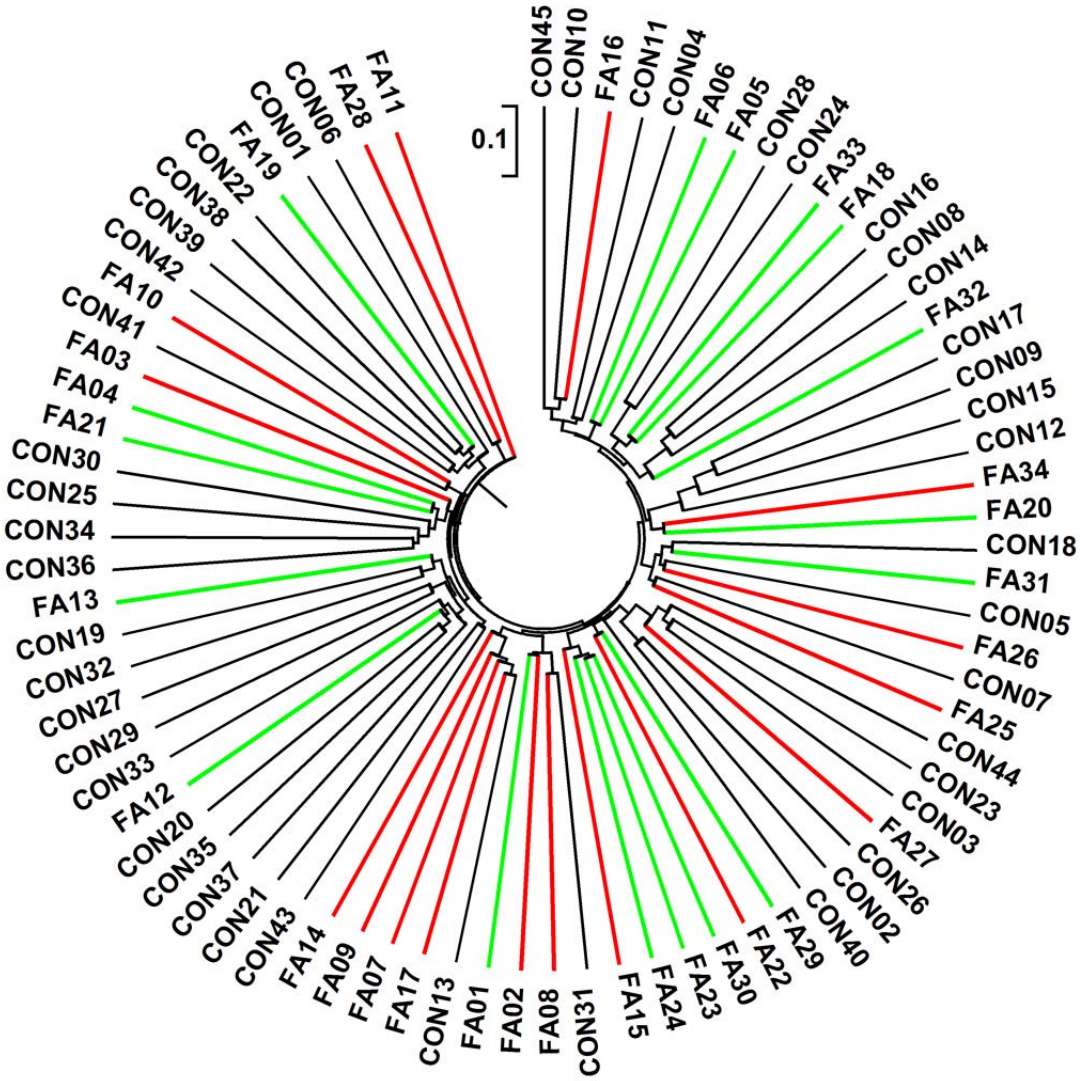
0.1
CON45
CON10
FA16
CON11
CON04
FA06
FA05
CON28
CON24
FA33
FA18
CON16
CON08
CON14
FA32
CON17
CON09
CON15
CON12
FA34
FA20
CON18
FA31
CON05
FA26
CON07
FA25
CON44
CON23
CON03
FA27
CON26
CON02
CON40
FA29
FA22
FA30
FA23
FA24
FA15
CON31
FA08
FA02
FA01
CON13
FA17
FA07
FA09
FA14
CON43
CON21
CON37
CON35
CON20
FA12
CON33
CON29
CON27
CON32
CON19
FA13
CON36
CON34
CON25
CON30
FA21
FA04
FA03
CON41
FA10
CON42
CON39
CON38
CON22
FA19
CON01
CON06
FA28
FA11

Figure S5

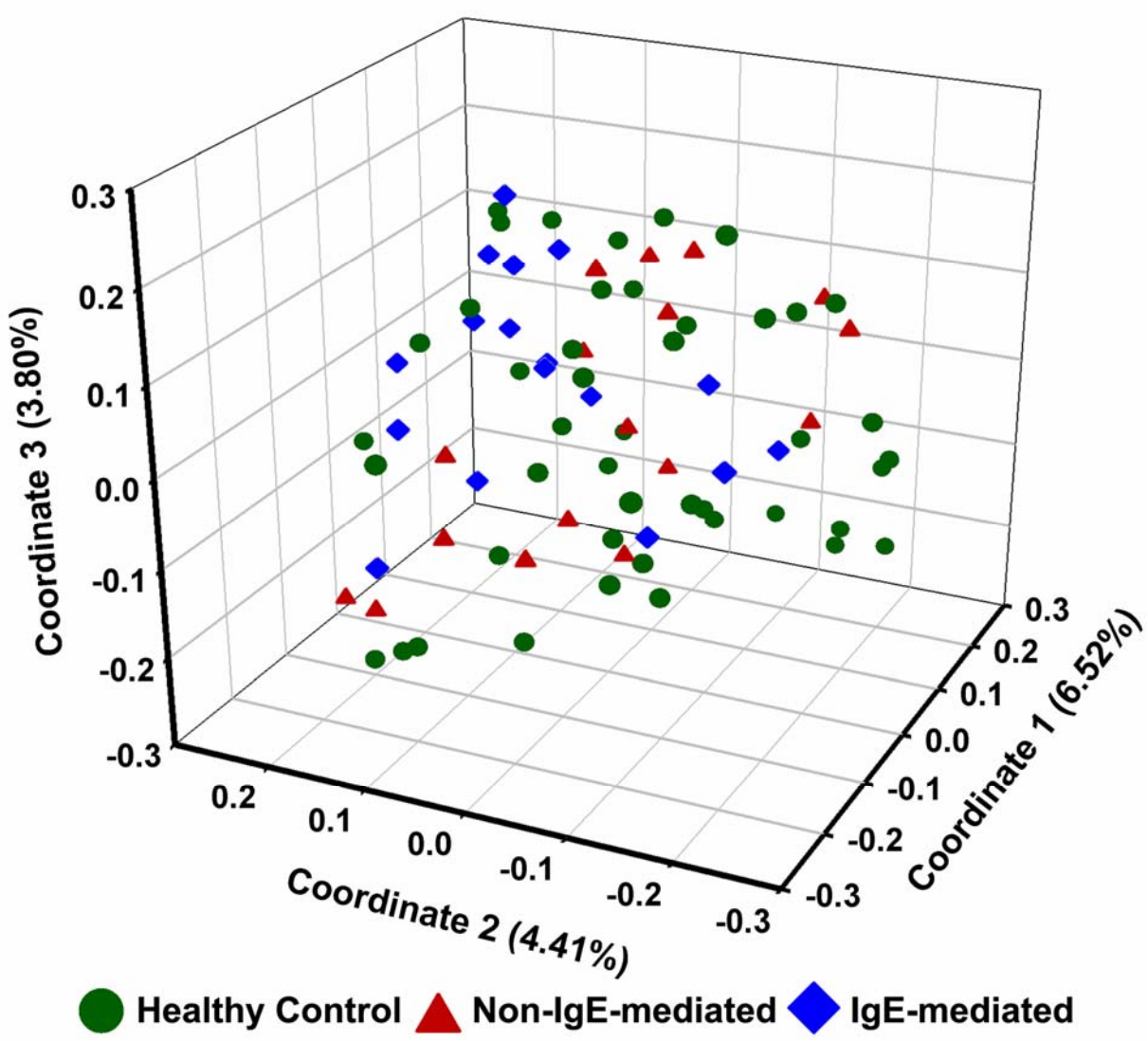
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
Coordinate 3 (3.80%)
0.2
0.1
0.0
-0.1
-0.2
-0.3
Coordinate 2 (4.41%)
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
Coordinate 1 (6.52%)
Healthy Control
Non-IgE-mediated
IgE-mediated

Figure S6

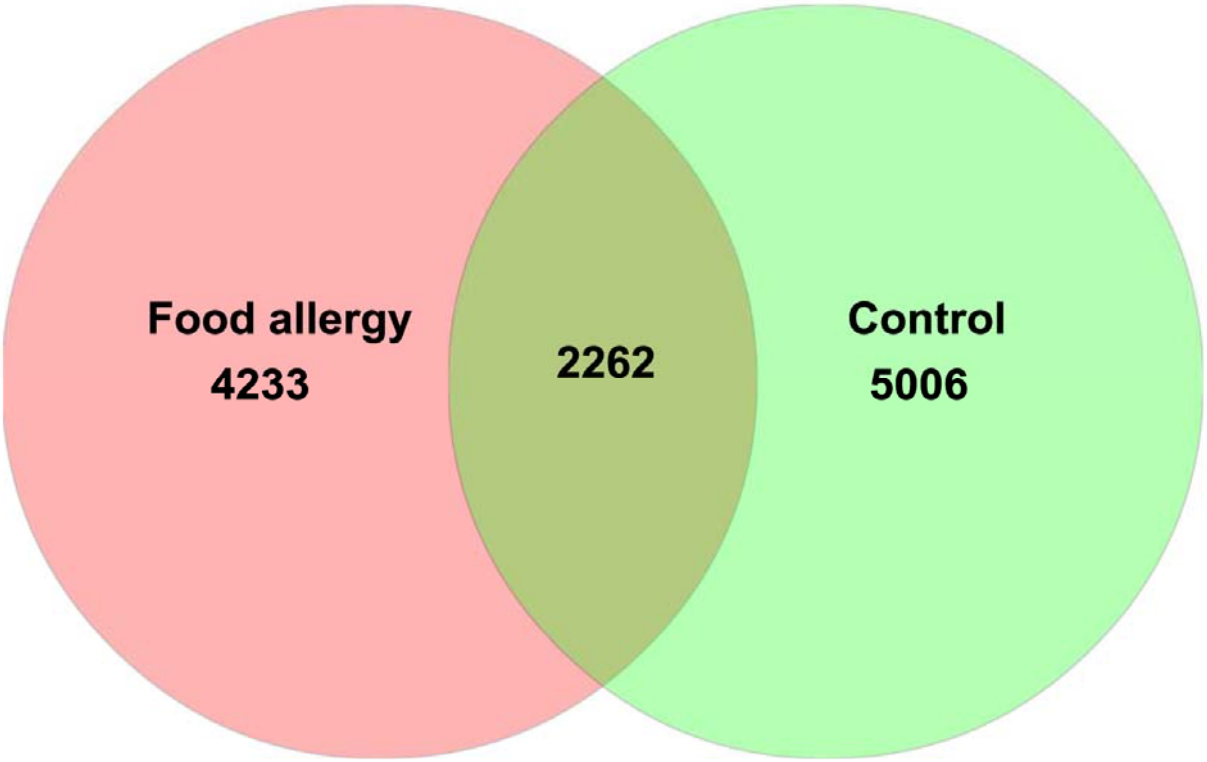

Figure S7

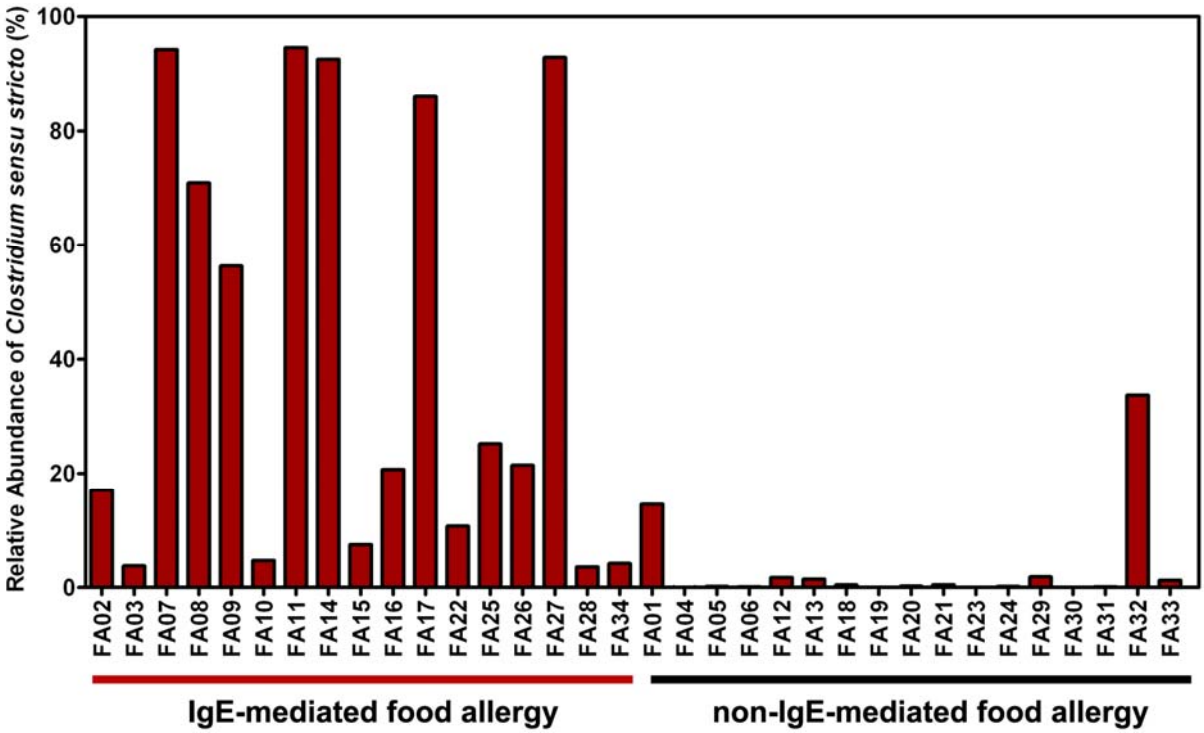
Relative Abundance of Clostridium sensu stricto (%)
100
80
60
40
20
0
FA02
FA03
FA07
FA08
FA09
FA10
FA11
FA14
FA15
FA16
FA17
FA22
FA25
FA26
FA27
FA28
FA34
FA01
FA04
FA05
FA06
FA12
FA13
FA18
FA19
FA20
FA21
FA23
FA24
FA29
FA30
FA31
FA32
FA33
IgE-mediated food allergy
non-IgE-mediated food allergy

Table S1 List of the 79 10-bp barcodes used to tag each PCR product analyzed as part of the study

Sample ID	Barcode	Sample ID	Barcode
FA01	CTCTACAGCG	CON07	ATCTGAGACG
FA02	CTGTCGTGCG	CON08	ATGCTACGTC
FA03	CTGTGACGTG	CON09	ATGTGACTAC
FA04	GACGCTGTCG	CON10	CACGAGACAG
FA05	GACGTATGAC	CON11	CACGCGAGTC
FA06	GACTAGCTAG	CON12	ACTCGCGTCG
FA07	GAGACGTCGC	CON13	CACGCTACGA
FA08	GAGAGAGACG	CON14	AGACTCGACG
FA09	GCGTAGACTA	CON15	CACGTGTATA
FA10	GCTCTCTACG	CON16	CACTACGATG
FA11	GTACACTGTA	CON17	CACTATACTC
FA12	GTACGCGACA	CON18	CAGCGTACTG
FA13	GTACTATAGA	CON19	CAGTCTCTAG
FA14	GTACTGAGTC	CON20	CATAGTCGCG
FA15	GTAGCTAGCG	CON21	CGAGACACTA
FA16	GTAGTCACTG	CON22	CGAGAGTGTG
FA17	GTAGTGTCAC	CON23	CGAGTCATCG
FA18	GTATACATAG	CON24	CGATCGTATA
FA19	GTCATCGTCG	CON25	CGCAGTACGC
FA20	GTCGACACGC	CON26	CGCGATCGTA
FA21	GTCGAGTGAG	CON27	CGCGCTATAC
FA22	GTCTACTATC	CON28	AGTACGAGAG
FA23	GTGTCTAGAC	CON29	CGTACAGATA
FA24	GTGTGTATCG	CON30	CGTAGCTCTC
FA25	ACACGACGAC	CON31	CGTATAGTGC
FA26	ACACGTAGTA	CON32	CGTCAGCGAC
FA27	ACACTACTCG	CON33	CGTCGCAGTG
FA28	ACGACACGTA	CON34	CGTCTCACGA
FA29	ACGAGTAGAC	CON35	CGTGACTCAG
FA30	ACGCGTCTAG	CON36	CTACACGCTC
FA31	ACGTACACAC	CON37	CTACGATATG
FA32	ACGTACTGTG	CON38	CTAGACAGAC
FA33	ACGTAGATCG	CON39	CTAGTACTCA
FA34	ACTACGTCTC	CON40	CTATATGTCG
CON01	ATAGAGCTAG	CON41	CTATCGACAC
CON02	ATATAGAGTA	CON42	CTATGTAGAG
CON03	ATCGCTCACG	CON43	CTCACGTACA
CON04	ATCGTCAGTC	CON44	CTCGAGTCTC
CON05	ACTATACGAG	CON45	CTCGTCGAGA
CON06	ATCTCTCGTA		

Table S2 Comparison of phylotypes coverage and diversity estimation of the 16S rRNA gene libraries for individuals at 97% similarity from the pyrosequencing analysis

Sample	Reads	OTUs	Good's	ACE	Chao 1	Shannon	Simpson
FA01	7894	282	97.75%	1249	708	2.4839	0.1882
FA02	9271	273	98.23%	1115	564	2.1561	0.2956
FA03	9734	331	98.04%	1186	701	2.7608	0.1332
FA04	6023	562	94.95%	1452	1193	4.1087	0.0479
FA05	9172	252	98.22%	1098	640	2.4762	0.1392
FA06	9546	204	98.84%	593	415	2.4291	0.1876
FA07	12851	213	99.12%	557	405	0.7498	0.7998
FA08	9153	370	97.40%	1957	1075	2.3229	0.3215
FA09	9029	254	98.14%	1295	692	2.1876	0.1900
FA10	9272	444	96.95%	2070	1258	2.9092	0.1468
FA11	13021	354	98.23%	1408	828	0.6154	0.8596
FA12	9504	300	98.32%	982	565	2.8613	0.1202
FA13	6716	339	97.04%	1238	777	3.3446	0.0844
FA14	10172	424	97.25%	2493	1205	2.4862	0.2272
FA15	11550	232	98.92%	740	467	1.6382	0.4904
FA16	8513	232	98.44%	824	598	1.5972	0.4395
FA17	10612	247	98.82%	553	468	1.6612	0.4522
FA18	11996	271	98.80%	722	495	1.9684	0.3481
FA19	10214	296	98.43%	745	573	1.8843	0.3614
FA20	15596	210	99.28%	557	364	0.9454	0.6054
FA21	8559	445	97.29%	1146	864	3.9579	0.0409
FA22	8530	317	97.66%	1358	841	2.7586	0.1415
FA23	9653	246	98.54%	781	586	1.6405	0.4214
FA24	11335	299	98.48%	998	619	1.9241	0.3146
FA25	8046	299	97.55%	2171	989	3.0146	0.0938
FA26	9993	267	98.46%	810	554	2.4498	0.1515
FA27	12152	241	98.68%	1193	730	0.8035	0.7808
FA28	4031	113	98.61%	268	204	1.6401	0.3409
FA29	13369	340	98.59%	1121	749	2.6232	0.1809
FA30	8630	246	98.34%	777	545	2.0980	0.2381
FA31	8222	183	98.71%	638	382	2.4120	0.1399
FA32	10830	277	98.41%	879	697	2.2933	0.1752
FA33	11403	242	98.91%	610	480	1.9481	0.3215
FA34	12334	297	98.79%	698	612	2.0151	0.3361
CON01	7530	297	97.41%	1594	747	2.6497	0.1386
CON02	11615	368	98.02%	1622	865	2.8040	0.1157
CON03	11537	265	98.74%	712	547	1.7204	0.3325
CON04	7129	286	97.63%	1208	650	2.9835	0.1191
CON05	12840	241	98.95%	448	462	2.1425	0.1852
CON06	8227	107	99.30%	300	194	0.7495	0.7490
CON07	8976	204	98.71%	677	451	1.3170	0.5715

CON08	8454	295	97.92%	1053	630	1.8024	0.3287
CON09	9270	275	98.23%	750	572	1.9956	0.2519
CON10	8584	257	98.16%	1094	657	2.2970	0.1974
CON11	6934	265	97.74%	1053	625	2.7636	0.1322
CON12	10300	240	98.64%	702	439	2.2481	0.1616
CON13	9264	306	97.94%	1126	718	2.1350	0.2386
CON14	11258	249	98.66%	860	539	1.9881	0.2215
CON15	8549	319	97.56%	1248	837	2.5908	0.1439
CON16	8712	228	98.30%	1154	539	2.0375	0.2325
CON17	6978	265	97.52%	1051	703	2.0383	0.2864
CON18	9823	186	98.95%	519	355	1.5704	0.4401
CON19	8726	220	98.71%	547	438	2.2071	0.2334
CON20	9912	301	98.38%	835	601	2.8027	0.1323
CON21	11578	179	99.33%	374	296	1.5564	0.4987
CON22	9648	247	98.75%	623	449	2.2457	0.2929
CON23	13056	239	98.98%	702	476	1.0206	0.6678
CON24	10076	294	98.42%	861	536	2.8797	0.1041
CON25	6558	444	96.19%	1495	1079	4.0590	0.0400
CON26	13741	302	98.76%	908	730	1.7628	0.3232
CON27	9464	323	97.87%	1696	903	3.2146	0.0835
CON28	10456	284	98.45%	938	767	2.6186	0.1498
CON29	12631	341	98.50%	1276	779	2.8830	0.1533
CON30	10380	334	98.44%	763	618	2.6092	0.1857
CON31	10778	461	97.49%	1622	1007	2.6566	0.2352
CON32	10431	323	98.26%	1080	720	2.2482	0.2882
CON33	9416	388	97.49%	1478	954	2.8757	0.1568
CON34	9892	496	97.22%	1887	1050	3.9979	0.0534
CON35	10762	325	98.47%	814	633	2.9035	0.1218
CON36	7697	322	97.77%	976	656	3.1291	0.1263
CON37	9100	320	97.79%	1757	878	3.1819	0.0815
CON38	9211	156	99.16%	351	283	1.2213	0.5708
CON39	8858	381	97.53%	1255	772	2.6568	0.1652
CON40	8866	314	97.89%	1486	760	3.1390	0.0989
CON41	10888	208	99.03%	547	440	1.6200	0.4921
CON42	11085	281	98.40%	1376	661	1.4205	0.5577
CON43	5808	323	96.87%	1068	756	2.7679	0.2132
CON44	6268	202	97.91%	917	506	2.0833	0.2324
CON45	12288	268	98.81%	821	539	1.7970	0.3734

Abbreviations: FA, food allergy; CON, healthy control.

Table S3 Differences in bacterial abundances between infants with and without food allergy in the phylum level

Phylum	Healthy Control		Food Allergy		Metastats P value	Metastats Q value[2]
	Mean	SE[1]	Mean	SE		
Firmicutes	65.1942%	0.0723%	70.9426%	0.0782%	0.0000E+00	0.0000E+00
Bacteroidetes	18.5105%	0.0590%	16.0376%	0.0632%	3.5967E-177	3.6137E-176
Proteobacteria	13.6629%	0.0522%	11.7269%	0.0554%	2.7742E-140	2.0905E-139
Actinobacteria	1.2238%	0.0167%	1.1258%	0.0182%	8.0713E-05	3.4755E-04

[1] SE: standard error.

[2] A comparison of fecal microbiota between infants with and without food allergy was performed using Metastats. Q value indicates that the false discovery rate and that a lower value is generally preferred to avoid a false positive result.

Table S4 Differences in bacterial abundances between infants with and without food allergy in the genus level

Phylum	Genus	Healthy Control		Food Allergy		Metastats P value	Metastats Q value
		Mean	SE	Mean	SE		
Firmicutes	Clostridium sensu stricto	10.6847%	0.0469%	24.4033%	0.0740%	0.0000E+00	0.0000E+00
Bacteroidetes	Bacteroides	16.8892%	0.0569%	14.0645%	0.0599%	4.2370E-251	1.0356E-249
Firmicutes	Streptococcus	12.3256%	0.0499%	8.5618%	0.0482%	0.0000E+00	0.0000E+00
Firmicutes	Enterococcus	2.3616%	0.0231%	8.2707%	0.0475%	0.0000E+00	0.0000E+00
Firmicutes	Veillonella	13.5559%	0.0520%	7.5845%	0.0456%	0.0000E+00	0.0000E+00
Proteobacteria	Escherichia/Shigella	6.1238%	0.0364%	7.3779%	0.0450%	4.2412E-106	7.5065E-105
Proteobacteria	Klebsiella	3.6074%	0.0283%	3.1108%	0.0299%	7.1452E-33	8.1497E-32
Firmicutes	Lactobacillus	1.4427%	0.0181%	3.0401%	0.0296%	0.0000E+00	0.0000E+00
Firmicutes	Staphylococcus	0.4472%	0.0101%	2.2658%	0.0256%	0.0000E+00	0.0000E+00
Firmicutes	Faecalibacterium	1.5428%	0.0187%	1.8434%	0.0232%	2.1470E-24	2.1192E-23
Firmicutes	Clostridium XIVa	0.8797%	0.0142%	1.7906%	0.0228%	5.9084E-273	1.5163E-271
Firmicutes	Blautia	2.3702%	0.0231%	1.7137%	0.0224%	4.9362E-89	7.9174E-88
Firmicutes	Anaerostipes	0.0039%	0.0010%	1.5448%	0.0212%	0.0000E+00	0.0000E+00
Bacteroidetes	Prevotella	0.8899%	0.0143%	1.3923%	0.0202%	9.1911E-97	1.5218E-95
Firmicutes	Clostridium XVIII	0.5277%	0.0110%	1.2935%	0.0195%	1.8086E-284	4.8858E-283
Firmicutes	Flavonifractor	0.7967%	0.0135%	1.0029%	0.0172%	1.1204E-21	1.0649E-20
Firmicutes	Clostridium XI	1.2912%	0.0171%	0.6052%	0.0134%	1.4158E-199	3.0278E-198
Firmicutes	Lachnospiracea_incertae_sedis	1.5624%	0.0188%	0.5521%	0.0128%	0.0000E+00	0.0000E+00
Firmicutes	Megamonas	1.3119%	0.0173%	0.0217%	0.0025%	0.0000E+00	0.0000E+00
Firmicutes	Megasphaera	1.2344%	0.0168%	0.0157%	0.0022%	0.0000E+00	0.0000E+00