

ORIGINAL ARTICLE

Food Allergy & Anaphylaxis

Fecal microbiome signatures are different in food-allergic children compared to siblings and healthy children

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Abstract

Background: Intestinal microbes have been shown to influence predisposition to atopic disease, including food allergy. The intestinal microbiome of food-allergic children may differ in significant ways from genetically similar non-allergic children and age-matched controls. The aim was to characterize fecal microbiomes to identify taxa that may influence the expression of food allergy.

Methods: Stool samples were collected from children with IgE-mediated food allergies, siblings without food allergy, and non-allergic controls. Stool microbiome characterization was performed via next-generation sequencing (Illumina) of the V1V3 and V4 variable regions of the 16S rRNA gene. Bacterial diversity, evenness, richness, and relative abundance of the operational taxonomic units (OTUs) were evaluated using QIIME. ANOVA and Welch's *t* test were utilized to compare groups.

Results: Sixty-eight children were included: food-allergic (*n* = 22), non-food-allergic siblings (*n* = 25), and controls (*n* = 21). When comparing fecal microbial communities across groups, differences were noted in *Rikenellaceae* (*P* = .035), *Actinomycetaceae* (*P* = .043), and *Pasteurellaceae* (*P* = .018), and nine other distinct OTUs. Food-allergic subjects had enrichment for specific microbes within the *Clostridia* class and *Firmicutes* phylum (*Oscillobacter valericigenes*, *Lachnoclostridium bolteae*, *Faecalibacterium* sp.) compared to siblings and controls. Identification of *Clostridium* sp. OTUs revealed differences in specific *Clostridia* drive the separation of the allergic from the siblings and controls. *Alistipes* sp. were enriched in non-allergic siblings.

Conclusions: Comparisons in the fecal microbiome of food-allergic children, siblings, and healthy children point to key differences in microbiome signatures, suggesting the role of both genetic and environmental contributors in the manifestation of food-allergic disease.

KEYWORDS

environment, food allergy, genetics, microbiome, T regulatory cells

1 | BACKGROUND

Recent research has focused on the role of the intestinal microbiome and its influence on the risk for atopic disease including food allergy

and eczema.^{1,2} The abundance, diversity, and metabolic activity of gut microbiota play an important role in the maintenance of homeostasis in a healthy state. The microbiome composition can shift secondary to environmental factors and is influenced by genetic risk

factors for disease. Early exposure to siblings or a large number of persons is a protective factor for allergic disease, with older siblings associated with increased richness and diversity of the microbiome,³ and a recent study reported clear differences in sibling microbiota with enrichment of *Moraxella*-dominant nasal microbiota profile and *Bifidobacterium*-dominant fecal microbiota profile.⁴ However, the composition of the microbiome in food-allergic patients and their siblings has not been explored. We hypothesize that the gut microbiome of food-allergic children is unique from their siblings and healthy controls.

Increasing evidence supports the idea that certain commensal bacterial species are protective and enhance gut barrier function, while the presence of other bacteria can increase inflammation.⁵⁻⁷ Commensal microbes contribute to enzyme digestion of complex carbohydrates, production of short-chain fatty acids (SCFAs), and biosynthesis of essential vitamins and hormones. In addition, the gut microbiome engages in competitive colonization, thereby providing protection against pathogen overgrowth.⁸ Rhoades et al demonstrated an increase in stool calprotectin (a marker of inflammation) correlating with symptoms of colic in infants and increased prevalence of intestinal *Klebsiella*.⁹ Experiments in mice have shown that specific *Clostridium* species as well as members of the *Bacteroides* can induce T regulatory cells, which are essential for immune tolerance.¹⁰ Enrichment of gut microbiota with certain microbial species is associated with the production of metabolites which can induce increased T-cell differentiation to a regulatory cell phenotype.¹¹ Distinct gut microbiome composition has been associated with milk allergy resolution, with *Clostridia/Firmicutes* enriched and *Bacteroidetes* underrepresented in children with milk allergy resolution versus milk allergy persistence at 8 years of age.¹² Cao et al recently published data indicating that commensal anaerobes in the GI tract prevent food allergen sensitization through the promotion of an IL-22-dependent barrier protective response limiting allergen entry into the blood stream.^{2,13} The protective effect of commensal bacteria on food allergen sensitization was further shown by Stefka et al.¹⁴

Gut microbiota are believed to play a vital role in early life immune development and function; a range of factors such as mode of delivery (vaginal versus C-section), antibiotic exposure, infant feeding practices (formula versus breastfeeding), and farming environment (the "hygiene hypothesis") have been associated with microbiome changes and risk of allergic disease.¹⁵

Some data show that probiotic treatment of pregnant and nursing women can reduce the risk of eczema in infants, suggesting an influence of the maternal microbiome on the development of atopy in the child.¹⁶⁻¹⁷ The maternal microbiome has been shown to be the source of commensal bacteria transfer to the infant through entero-placental and entero-mammary circulation, as well as through direct skin to skin and mucosal contact.¹⁸ Studies have shown that supplementation of extensively hydrolyzed casein infant formula with *Lactobacillus* GG may accelerate the development of tolerance to cow's milk protein.¹⁹ Dietary manipulation can also influence the microbiome in infants.²⁰

Medications that alter the microbiome have also been shown to alter food allergy risk and natural history. Antibiotic treatment during infancy, which can alter the prevalence of certain commensal bacteria in the gut microbiome, increases the risk of food allergy in a dose-response fashion. Infants exposed to any antibiotics have increased risk of food allergy development with an odds ratio of 1.71 compared to unexposed cohorts, while those who had more than 4 courses of antibiotics are at even greater risk, with an odds ratio of 2.15.²¹

Although a causal link has not been established, proton pump inhibitor (PPI) use has been shown to predispose to food allergy development through proposed mechanisms of impairing full protein digestion or by increasing intestinal permeability.²² Recent research shows that daily use of a PPI is associated with significant alterations in the healthy fecal microbiome.²³

There is clear clinical benefit to be gained by further exploration and characterization of the differences between the microbiome of food-allergic patients and those without food allergy in order to drive future research toward therapeutic options. Reducing the burden of food allergy through manipulation of the microbiome could substantially improve the quality of life of children and families. Further elucidation of the role of the gut microbiome in the development and modulation of food allergy may, in the future, impact care of food-allergic patients.

We hypothesize that (i) the intestinal microbiome of food-allergic children will differ in significant ways from genetically similar children without food allergy (siblings raised in the same households) and (ii) the intestinal microbiome of food-allergic children will differ significantly from that of age-matched healthy controls without food allergy.

This case-control study of food-allergic children, their siblings, and age-matched healthy control children characterized the differences between gastrointestinal microbiomes to identify taxa that may influence the expression of food allergy, thereby exploring the genetic versus environmental contributors in the manifestation of food-allergic disease.

2 | METHODS

Food-allergic subjects and their non-food-allergic siblings were recruited from the Allergy & Immunology Clinic of Texas Children's Hospital. Children from birth to 18 years of age with physician-diagnosed food allergy (confirmed with a history of allergic reaction to a specific food and immediate hypersensitivity skin testing positive to that food and/or positive food-specific IgE) were eligible for participation. Non-allergic, healthy controls recruited from the community were utilized for comparison. Children with neurodevelopmental disabilities, significant anatomic abnormalities of the stomach or intestines, prematurity <35 weeks gestation and long-term TPN therapy were excluded.

Historical data pertaining to body mass index (BMI), breastfeeding, mode of delivery, specific antibiotic use before 2 years of age,

use of PPI therapy for gastroesophageal reflux (GERD), and history of probiotic treatment were obtained in all food-allergic and sibling participants through a survey (see Supporting Information). This information was also obtained from healthy controls when possible. All subjects were recruited under a protocol approved by the Baylor College of Medicine Institutional Review Board.

A single stool specimen was collected from each subject, refrigerated, and transported to the Texas Children's Microbiome Center. Bacterial DNA was extracted using the MoBio Powersoil DNA kit (Mo Bio Laboratories, Inc., Solana Beach, CA), with minor modifications. Microbial DNA was quantitated by Nanodrop-1000 (Thermo Scientific, Waltham, MA) and Qubit (Life Technologies, Grand Island, NY). Amplification and next-generation sequencing targeting the V4 region of the 16S rRNA gene was performed using the NEXTflex™ 16S V4 Amplicon-Seq Kit 2.0 (Bioo Scientific, Austin, TX), and

sequences were generated on the Illumina MiSeq platform (Illumina, San Diego, CA) with a median of 10 640 sequences generated per sample. Sequence data were processed through the LotuS pipeline as previously described.²⁴ Briefly, reads were de-multiplexed and paired ends stitched. Quality filtering was performed prior to operational taxonomic unit (OTU) clustering utilizing a modified version of the UPARSE algorithm.²⁵ Taxonomic assignment was performed with RDP as the classifier and SILVA²⁶ as the selected database. Organisms potentially classified to the species level were based on individual OTUs of significance. OTUs failing to classify as bacteria at the kingdom level were removed prior to further analysis of the dataset. Bacterial diversity, evenness, richness, and relative abundance of the OTUs identified in each sample were evaluated using QIIME²⁷ ANOVA and Welch's *t* test within STAMP²⁸ were utilized to compare between groups. Age comparisons were performed

TABLE 1 Baseline Demographics and Clinical Characteristics

Characteristic	Study groups			P-values		
	Food allergic (n = 22)	Siblings (n = 26)	Healthy controls (n = 20)	Healthy control vs food allergic	Healthy control vs sibling	Food allergic vs sibling
Age (n)						
<7y	9	8	9	NS*	NS	NS
7-18y	13	18	11	NS	NS	NS
Sex (n)						
Female	10	14	12	NS	NS	NS
Male	12	12	8	NS	NS	NS
BMI (n)			[ND = 3]**			
<85%	15	22	15	NS	.04	.044
≥85%	7	4	2	.024	NS	NS
Antibiotics (n)	[ND = 2]	[ND = 4]	[ND = 6]			
Yes	14	17	9	NS	NS	NS
No	8	5	2	NS	NS	NS
GERD (n)	[ND = 1]		[ND = 8]			
Yes	4	4	2	NS	NS	NS
No	18	22	10	NS	.02	NS
Breastfed (n)			[ND = 6]			
Yes	21	21	14	.03	.03	NS
No	1	5	0	NS	.001	.001
Prematurity (n)			[ND = 1]			
Yes	1	5	2	NS	NS	.01
No	21	21	17	NS	NS	NS
Delivery mode (n)			[ND = 6]			
Vaginal	13	15	9	.04	.02	NS
Cesarean	9	11	5	NS	.01	NS
Probiotics (n)			[ND = 1]			
Yes	2	1	1	NS	NS	NS
No	20	25	18	NS	NS	NS

*NS = Not significant. *P* > .05 (ANOVA followed by nonparametric post hoc test).

**ND = No data.

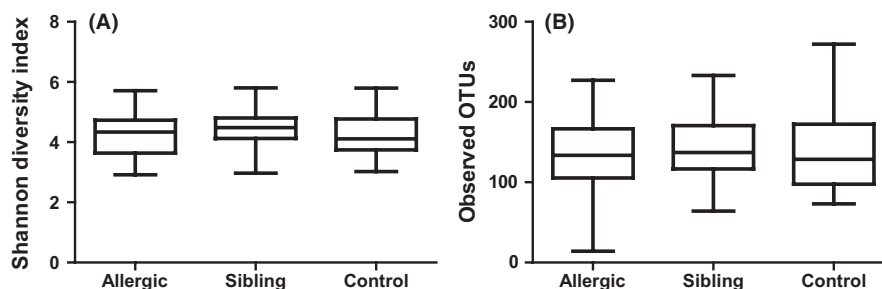


FIGURE 1 Alpha diversity for the allergic, sibling, and control subjects is similar across groups. A, The calculated Shannon diversity index for the three groups demonstrates the similar bacterial diversity found among all subjects. B, The total number of observed operational taxonomic units (OTUs) in each individual sample was also compared between the groups, and there was no difference in the amount of OTUs observed in the three groups

in subjects less than 7 years of age compared to older subjects. Multiple test correction was not performed due to the small number of subjects in each group.

3 | RESULTS

A total of 68 children were included in the comparison of the food-allergic ($n = 22$), non-food-allergic sibling ($n = 25$), and healthy

control ($n = 21$) groups. Age- and sex-matched controls were selected for each of the food-allergic participants (Table 1). The groups did not differ from each other with regard to BMI, use of antibiotics, and use of probiotics. The food-allergic children did not differ from the control and sibling group with regard to GERD, but the sibling group had slightly higher proportion of GERD than the healthy controls. The majority of all groups were breastfed, but the controls were not breastfed as frequently (70%) as the food-allergic children and siblings (>81%). The sibling group was more likely to have been born premature (19%) compared to their food-allergic siblings (5%). Delivery mode was not different between food-allergic children and their siblings. The food allergies noted in the food-allergic group included peanut/tree nuts ($n = 20$), fish ($n = 3$), milk ($n = 5$), egg ($n = 6$), sesame ($n = 4$), and soy ($n = 2$) (Table 2). One patient in the food-allergic group each had allergies to wheat, chick pea, lentil, avocado, and green pea, and within the healthy control group, four patients had antibiotic allergy.

Bacterial diversity based on the Shannon index and observed number of OTUs was similar between all three groups (Figure 1). When comparing the food-allergic group to the control group, a decrease in the Pasteurellales ($P = .032$) was seen in the food-allergic group, with a total of five OTUs differing between the groups. When comparing microbial communities across all three groups, differences were seen in *Rikenellaceae* ($P = .035$), *Actinomycetaceae* ($P = .043$), and *Pasteurellaceae* ($P = .018$), as well as in nine distinct OTUs. Subtle differences were visible at the phylum level, with greater resolution of key differences at the genus level (Figure 2). The *Clostridium* OTUs were further identified to the species level, showing changes in specific *Clostridium* drive the separation of the allergic group from the control and sibling groups.

Several bacteria associated with the food-allergic group were identified, with increased relative abundance of several OTUs observed in the food-allergic group as compared to the sibling and control groups. Statistically significant differences were determined in *Oscillobacter valericigenes*, *Lachnoclostridium bolteae*, and *Faecalibacterium* sp. The sibling group displayed intermediate abundance (decreased compared to allergic but increased compared to controls) to these specific bacteria (Figure 3). The increase in *O. valericigenes* was most noted in children >7 years

TABLE 2 Atopy profile across study groups

Study groups			
Atopy	Food allergic	Siblings	Healthy controls
Food allergy (n)			
Nuts	20	0	0
Fish	3	0	0
Milk	5	0	0
Eggs	6	0	0
Sesame	4	0	0
Wheat	1	0	0
Soy	2	0	0
Breast milk	1	0	0
Chickpea	1	0	0
Lentil	1	0	0
Avocado	1	0	0
Pea	1	0	0
Other (n)			
Atopic rhinitis	17	7	0
Eczema	9	1	0
Asthma ^{a,b}	11	3	0
Antibiotic	1	0	4
Latex	1	0	0

^aThere was no patient on oral immunotherapy or biologic therapy for allergic disease during this study.

^b5 food allergic patients and 2 siblings were on inhaled steroids at the time of this study.

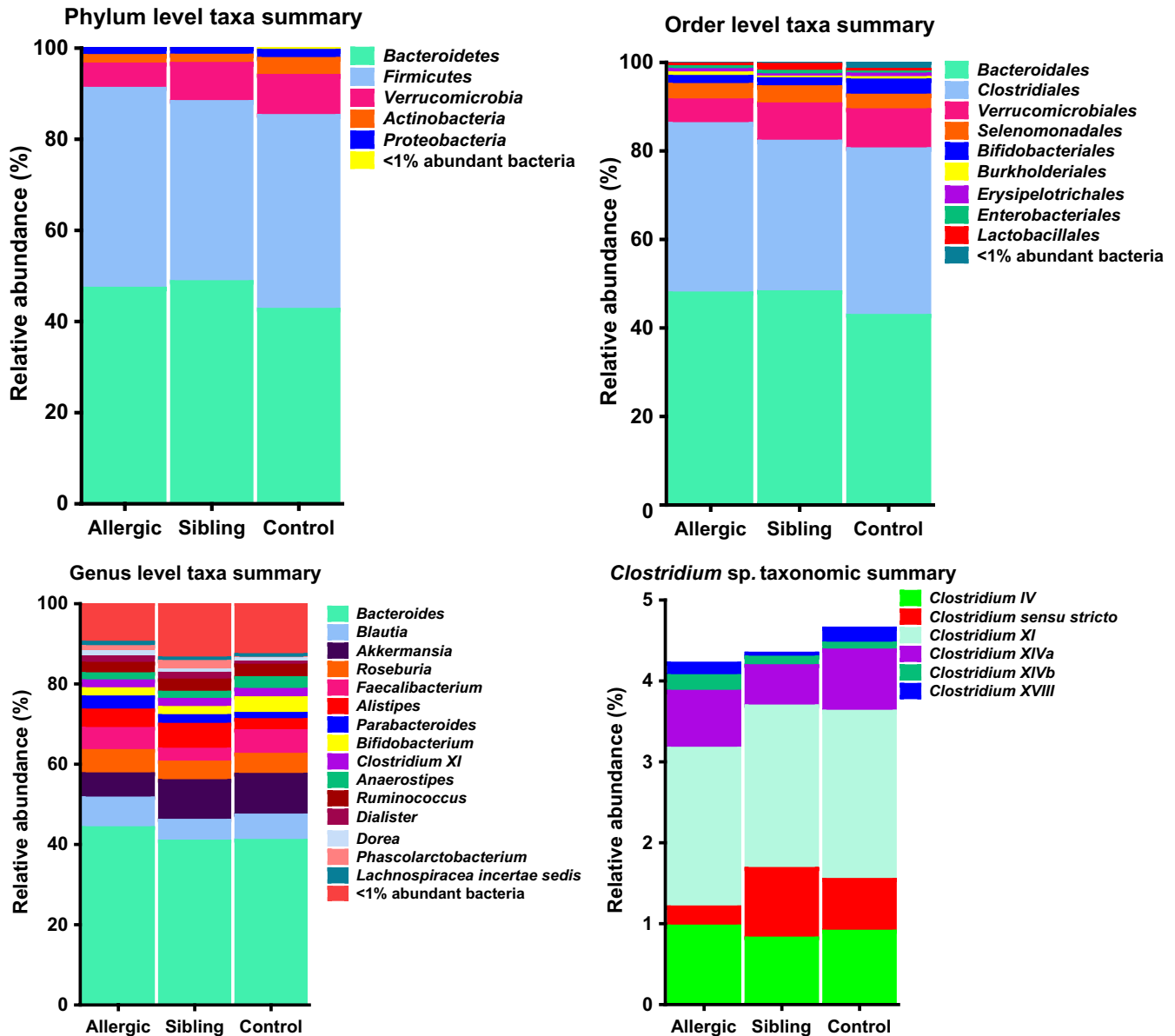


FIGURE 2 Microbiome profiles per group (allergic, sibling, and control) show subtle differences in overall composition. These differences in the gut microbiome were visible at the phylum level (left panel); however, a greater differentiation between the groups was observed when comparisons were made at the order (second panel) and genus level (right panel). The third panel depicts the *Clostridium* operational taxonomic units that were further identified to the species level. Some of the changes in specific *Clostridium* drive the separation of the allergic group from the control group

old, while the *L. bolteae* was more abundant in children <7 years (Figure S1). *Haemophilus parainfluenzae* showed a non-significant trend and *Blautia* sp. was identified with statistically significant decreased relative abundance in both the food-allergic and sibling groups compared to healthy controls (Figure 4). The decreased *Blautia* sp. was most prominent in children <7 years old (Figure S2). Unexpectedly, the non-allergic sibling group manifested a unique microbiome that, while drifting toward the allergic microbial profile, also displayed differences unique to the sibling group including increases in *Alistipes* sp. when the calculated ANOVA three-group comparison was performed. *Alistipes putredinis* and another *Alistipes* were more abundant in siblings of food-allergic

children than controls (Figure 5). This was most strongly noted in the older age group (Figure S3).

When evaluating group differences within each of the age ranges, variable patterns emerged based on age. In the <7 years cohort, *Verrucomicrobia* was increased in the controls compared to the allergic and sibling groups (Figure 6). Differences were also observed at the genus level, with increases and decreases in *Clostridium* contributing to the separation between the age groups. Additional observations included a greater abundance of *Actinomyces* ($P = .035$), *Akkermansia* ($P = .00856$), and *Peptostreptococcus* ($P = .047$) in the control group, increased *Parabacteroides* ($P = .046$) in the allergic group, and increased *Helicobacter* ($P = .041$) in the sibling group, with

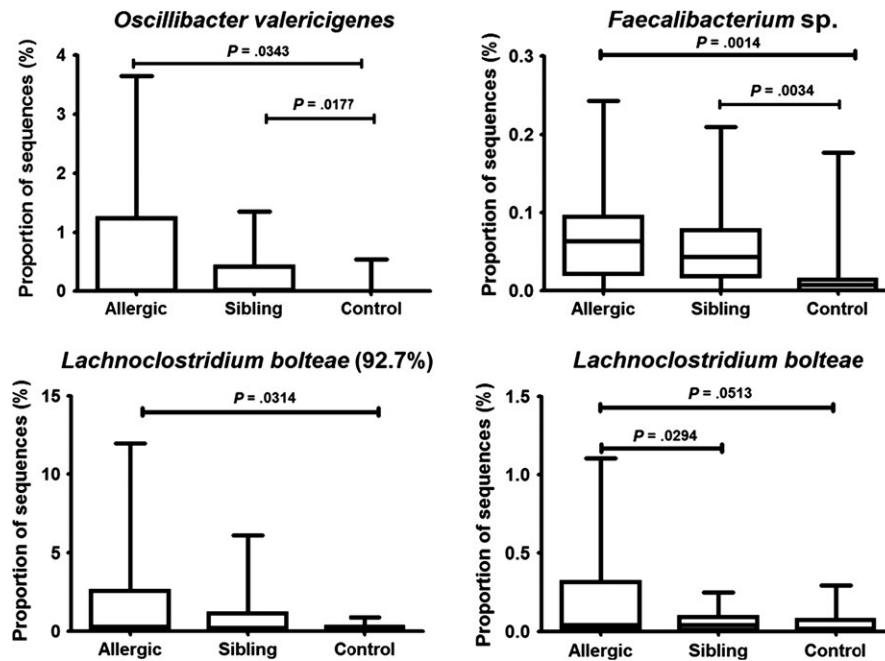


FIGURE 3 Several bacteria associated with food allergies were identified. Increased relative abundance of several operational taxonomic units (OTUs) was observed in the food-allergic group as compared to the sibling and control groups. Statistically significant differences were determined by a two-tailed Mann-Whitney test, in OTUs identified as *Oscillibacter valericigenes*, *Lachnoclostridium bolteae*, and *Faecalibacterium sp.* The ANOVA comparison of all three groups for *O. valericigenes*, *Faecalibacterium sp.*, *L. bolteae* (92.7%) and *L. bolteae* is $P = .007$, $P = .008$, $P = .036$, and $P = .017$, respectively. “*L. bolteae* (92.7)” represents an OTU whose representative sequence most closely matched to *L. bolteae* at 92.7% similarity. Of note, the sibling group displayed intermediate abundance (decreased compared to allergic but increased compared to controls) of several bacteria

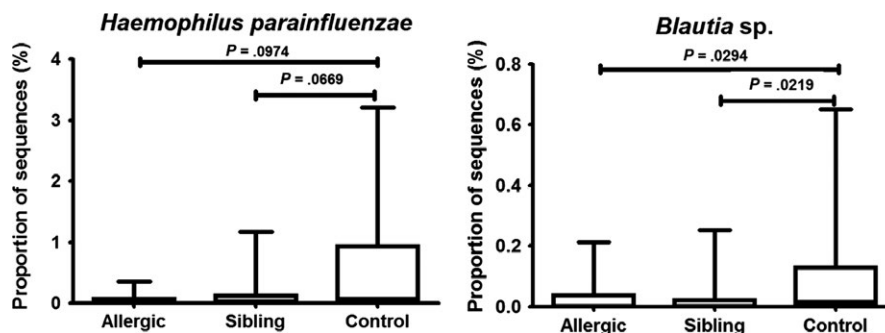


FIGURE 4 Decreased relative abundance of individual operational taxonomic units (OTUs) was observed in both the food-allergic and sibling groups compared to the control group. OTUs identified as *Haemophilus parainfluenzae* and *Blautia sp.* were observed predominantly in the control subjects rather than the allergic and sibling subjects. The reported P -values were determined by a two-tailed Mann-Whitney test. ANOVA comparison of the three groups (allergic, sibling, a control) yielded $P = .008$ for *H. parainfluenzae* and $P = .019$ for *Blautia sp.*

a total of 11 OTUs of significance in the three-group comparison. Fewer differences were observed within the 7-18 years group, with increased *Gemmiger* ($P = .045$) and *Lachnospiraceae incertae sedis* ($P = .031$) in the control group, with a total of 9 OTUs differing between groups.

When separating the groups by age range, high level differences revealed increased *Firmicutes* ($P = .026$) and *Proteobacteria* ($P = .029$) and decreased *Bacteroidetes* ($P = .035$) in the <7 years group compared to the 7-18 years group. The differences observed based on age did not overlap with the bacteria shown to differentiate between the allergic, sibling, and control groups, with increases

in *Erysipelotrichaceae* ($P = .00051$), *Lachnospiraceae* ($P = .025$), and *Peptostreptococcaceae* ($P = .018$) identified in the <7 years group. In addition, no marked differences were seen based on sex or BMI. Differences based on specific food allergen were not explored due to the small sample size and lack of power.

4 | DISCUSSION

The role of the microbiome in allergic disease is currently a topic of intense interest and research. There is increasing evidence that

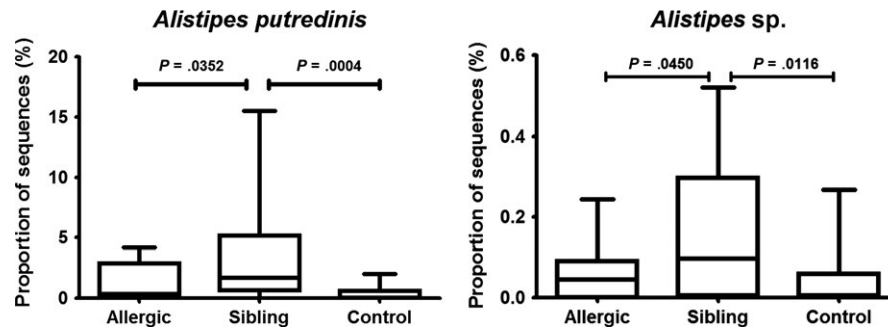


FIGURE 5 The sibling group displayed evidence of a unique gut microbiome. Increases in three organisms (operational taxonomic units [OTUs] identified as *Alistipes putredinis*, *Alistipes sp.*, and *Odoribacter splanchnicus*) were observed in the sibling group. While in greatest abundance in the sibling group, these OTUs were more closely associated with the food-allergic group rather than the control group. P-values were calculated using a Mann-Whitney, two-tailed test with no corrections. The calculated ANOVA three-group comparison for *A. putredinis*, *Alistipes sp.*, and *O. splanchnicus* was identified as $P = .01$, $P = .006$, and $P = .134$, respectively

microbial communities in the human body contribute significantly to the early development of the immune system affecting the balance between health and disease and influencing the development of allergy.²⁹ In this case-control study, we identify specific microbes that differentiate food-allergic children from their siblings as well as food-allergic children and siblings from age-matched healthy controls. This observation has potential implications for the role of both environmental and genetic influences on the fecal microbiome and the expression of food-allergic disease. In addition, our findings contribute to the future potential of gut microbiome characterization in a clinical setting. There were more differences between the groups in the younger age range (<7 years old), whereas the older 7- to 18-year-old food-allergic children, siblings, and controls were less distinct, supporting prior observation that changes in the microbial community structure early in life can effect later clinical disease phenotypes.³⁰

Food-allergic subjects had enrichment in the fecal microbiota for specific microbes within the *Clostridia* class and *Firmicutes* phylum (*O. valericigenes*, *L. bolteae*, *Faecalibacterium sp.*) compared to the non-food-allergic subjects. *L. bolteae* has previously been associated with dysbiosis potentially related to autism spectrum disorder, acute rejection of liver transplant, and decreased bacterial diversity.³¹ Non-food-allergic subjects also had enrichment of other *Clostridium* species (*Clostridia sensu stricto*). The differences in specific *Clostridium* species drove the separation between the food-allergic and non-food-allergic groups, indicating heterogeneity of function and disease association within the class. This observation highlights the importance of identifying specific species and their function in the evaluation of the role of the microbiome in atopic disease.

Alistipes putredinis and other *Alistipes sp.* were enriched in non-food-allergic siblings. These subjects had similar genetic risk factors and, presumably, could have developed food-allergic disease with the same environmental influences their sibling received. In a previous study of food sensitized children compared to controls, there was a decreased amount of *Alistipes sp.*³² This is consistent with the observation in our study of an increased amount of *Alistipes sp.* in the siblings who do not have food allergy. *Alistipes* may have a

protective function in allergic disease. Alternatively, it may be the result of a different diet in the food-allergic children compared to the non-food-allergic siblings. *Alistipes putredinis* has been shown to be induced in the gut microbiome of people eating a cruciferous vegetable diet (vegetables such as broccoli and cabbage) compared to a low fiber low-phytochemical diet (low fruit and vegetable).³³ In our study, the dietary habits of each child were not recorded, so this possibility could not be explored any further. In future studies, recording dietary habits will be important to include due to the influence of diet on fecal microbiome.

Blautia sp. were strongly enriched in the healthy control fecal samples from our cohort. *Blautia sp.* has been associated with increased butyrate production, a marker of tolerance in cow's milk allergy, and it was one of the strains that distinguished between cow's milk tolerant and allergic infants in a recent study. In an effort to therapeutically manipulate the microbiome (prevention or treatment of food allergy), the administration of probiotics has been investigated with *Lactobacillus rhamnosus* GG showing a positive effect on allergy resolution in milk-allergic infants.³⁴ *Blautia sp.* was enriched after treatment with extensively hydrolyzed cow's milk formula and *L. rhamnosus* GG in these infants.³⁴ This species association with healthy food tolerant subjects may be associated with an anti-inflammatory function that needs to be explored.

Interestingly, the other microbe with a trend toward differentiating controls from food-allergic subjects and siblings, *H. parainfluenzae*, is a potential pathogen that can trigger transforming growth factor β -activated kinase 1 (TAK1)/mitogen-activated protein kinase (MAPK) activation. This trigger can subsequently induce corticosteroid resistance (non-responsiveness to steroids) in asthma patients.³⁵ Decreased TAK1 activity causes inflammation in skin and intestinal tissue, indicating a key role of TAK1 for the maintenance of epithelium homeostasis.³⁶ Induction of TAK1/MAPK function by microbes may inhibit allergic disease expression and be implicated in food allergy.

There are several studies in germ-free mice that indicate gut microbial species influence the development of food allergen sensitization and disease. Mouse studies show that germ-free mice and

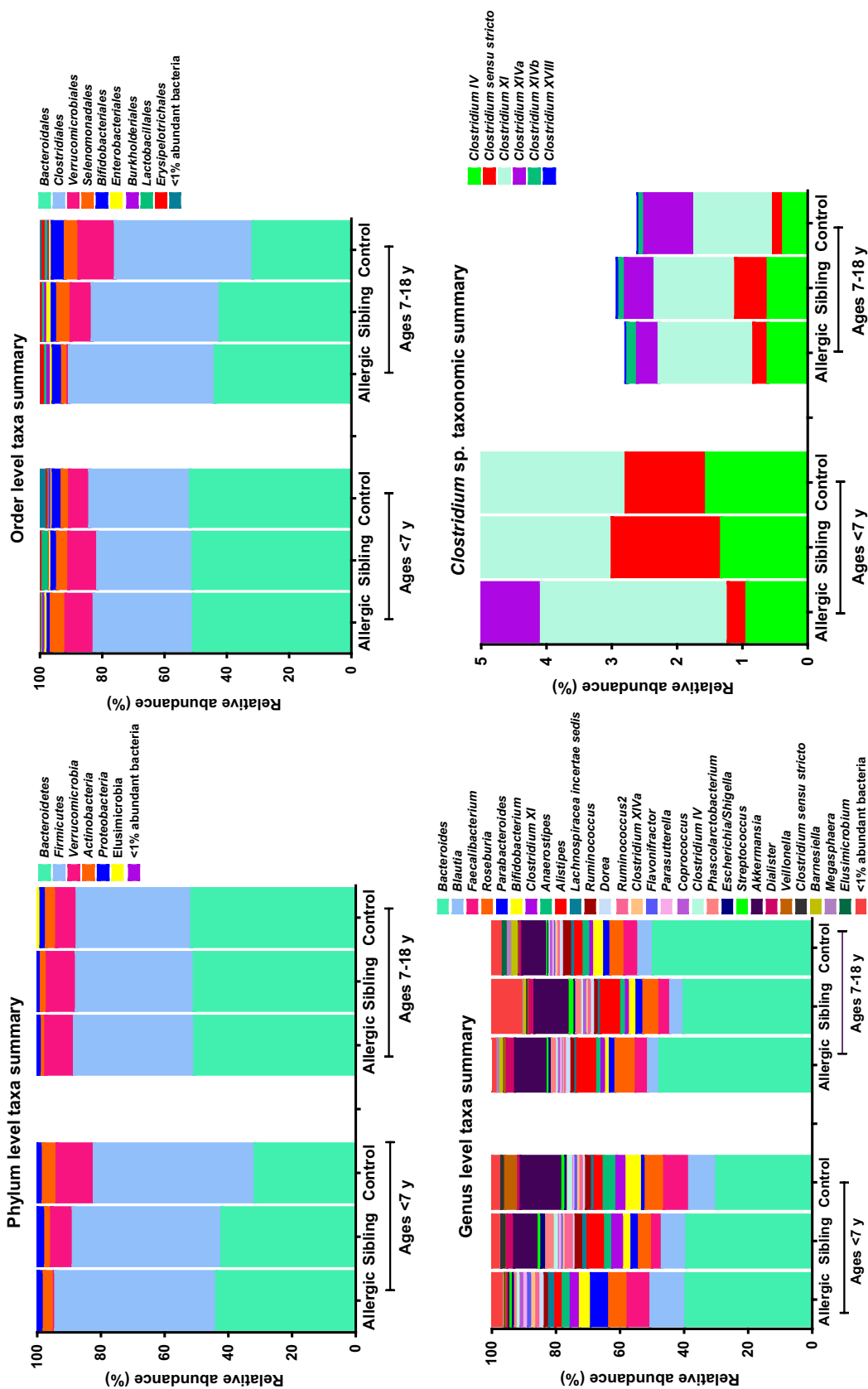


FIGURE 6 Microbiome profiles per group (allergic, sibling, and healthy controls separated by age) show subtle differences in overall composition at the order level. The separation of the groups is highly driven by both increases and decreases in specific *Clostridium* sp. when comparing the two age groups

those with low bacterial diversity develop serum IgE levels in early life. There is a critical level of microbial diversity after birth in murine neonates to induce inhibition of IgE production through an immunoregulatory network.³⁷ Commensal bacteria can protect against food sensitization in murine models of peanut allergy. Clostridial species induce IL-22 which regulates the movement of ingested peanut allergen to the systemic bloodstream, implicating microbial species as having an allergy protection capacity.¹⁴ T regulatory (Treg) cell induction and modulation of innate lymphoid cell function and intestinal epithelial permeability are proposed mechanisms of this capability.^{10,14} Ex vivo culture of human adult peripheral T cells with sterile fecal water from atopic subjects increased Treg cell abundance, implicating a metabolite which can directly induce Treg cells.¹¹

Signals derived from *Clostridia*, a class of anaerobic spore-forming *Firmicutes* that resides in close proximity to the colonic epithelium, are required in mice to prevent sensitization to food allergens. In addition to gut microbes protecting against allergic disease, bacterial metabolites, such as SCFAs, may contribute to the process through the ability to promote Treg differentiation.² Fecal butyrate producing *Clostridial* strains have been shown to be enriched in the colon, and butyrate production is considered a marker of intestinal health.³⁴ Non-food-allergic infants show increased fecal butyrate levels, and this may be a marker of microbial protection against intestinal permeability of food allergens and the development of food-allergic disease. Bacteria-produced SCFAs critically regulate both the proportions and functional capabilities of colonic regulatory T cells,^{38,39} which can be manipulated by dietary changes.

Early observations have suggested that an increased number of siblings in a family may protect children from allergic disease, whereas a clean, westernized lifestyle has been associated with a higher prevalence of allergic disorders ("the hygiene hypothesis"). A recent study investigated the prevalence of asthma between children of two very different farming populations (and differing microbial exposures) but with very similar genetic ancestries and lifestyle (Amish and Hutterites). It showed that Amish schoolchildren who practiced traditional farming and were exposed to an environment rich in microbes demonstrated very low rates of asthma, compared with Hutterite school children who used industrialized farming practices and had much less microbial exposure.⁴⁰ These findings reiterate the important role of the microbiome and environmental exposure in the development of allergic disease in people with genetically similar background.

To our knowledge, this is the first study of the fecal microbiome in food-allergic subjects to include a sibling group and non-allergic controls. The primary aim of our work was to investigate and characterize differences in the microbiome of food-allergic, non-food-allergic siblings and non-allergic children (controls). These comparisons point to key differences in microbiome profiles of allergic children and those from a healthy normal population. Another important contribution of this work is to define the gut microbiome of non-allergic siblings. Once the differences between the microbiome of food-allergic patients and those without allergy are further characterized in larger populations, we can use this knowledge to design

therapeutic approaches that may prevent the development of food allergies through specific probiotic supplementation in mothers, infants, and children and to glean mechanistic insights using metabolomics approaches. We may further be able to use this knowledge to design therapeutic studies aimed at modulating the severity and symptomology of food allergy.

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CONFLICT OF INTEREST

No authors have a conflict of interest for this work.

AUTHOR CONTRIBUTIONS

Dr. Kourosh contributed to the design of the study, data collection, data generation, interpretation, and preparation of the manuscript. Drs. Devaraj, Luna, and Balderas contributed to data generation, analysis, interpretation, and preparation of the manuscript. Drs. Anagnostou, Devaraj, and Nance contributed to the preparation of the manuscript and critical revision of the manuscript. Dr. Davis contributed to the conception and design of the study, data collection, generation, interpretation, and preparation and critical revision of the manuscript.

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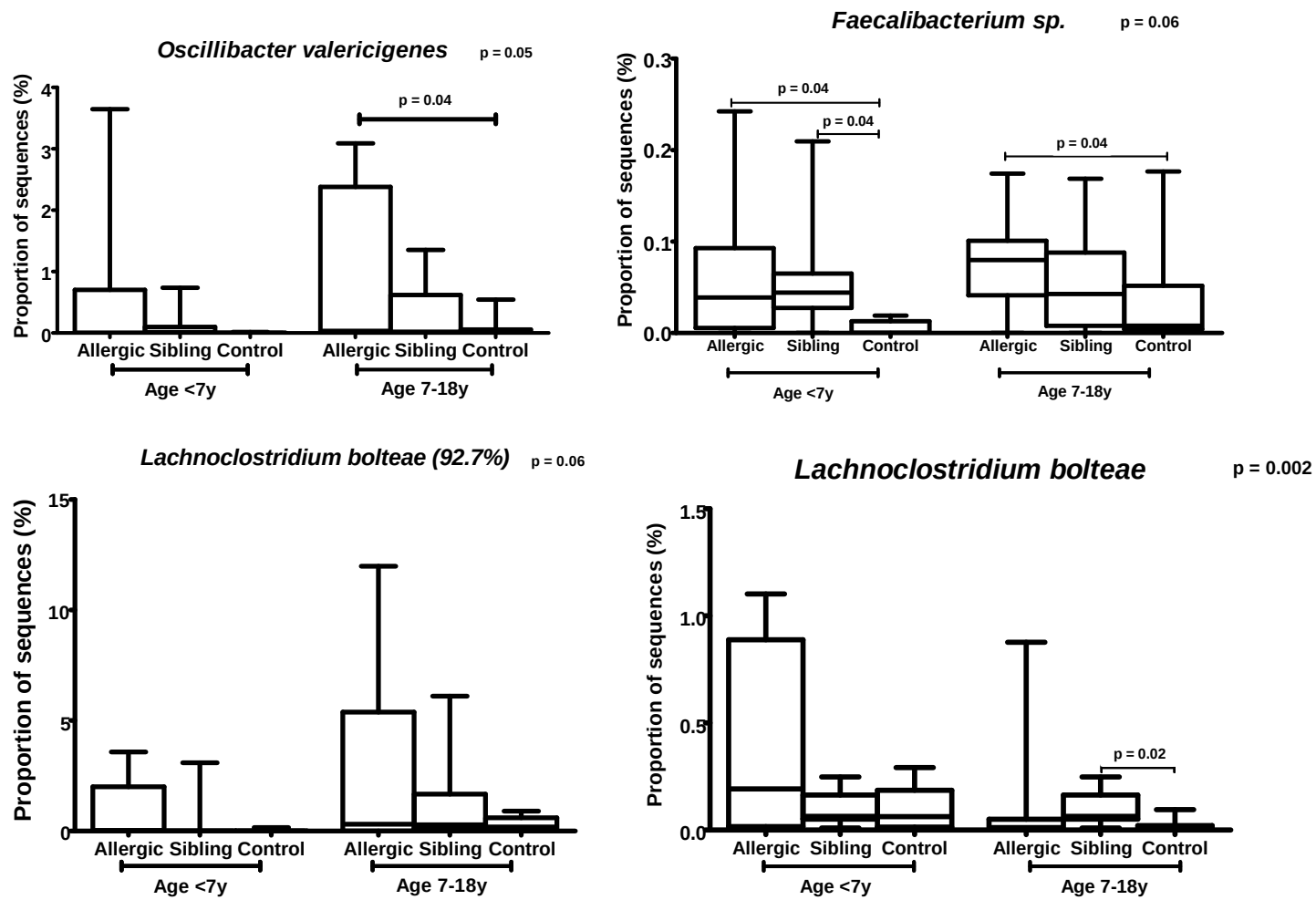
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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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1 Figure 1. Supplemental



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6 Figure 2. Supplemental

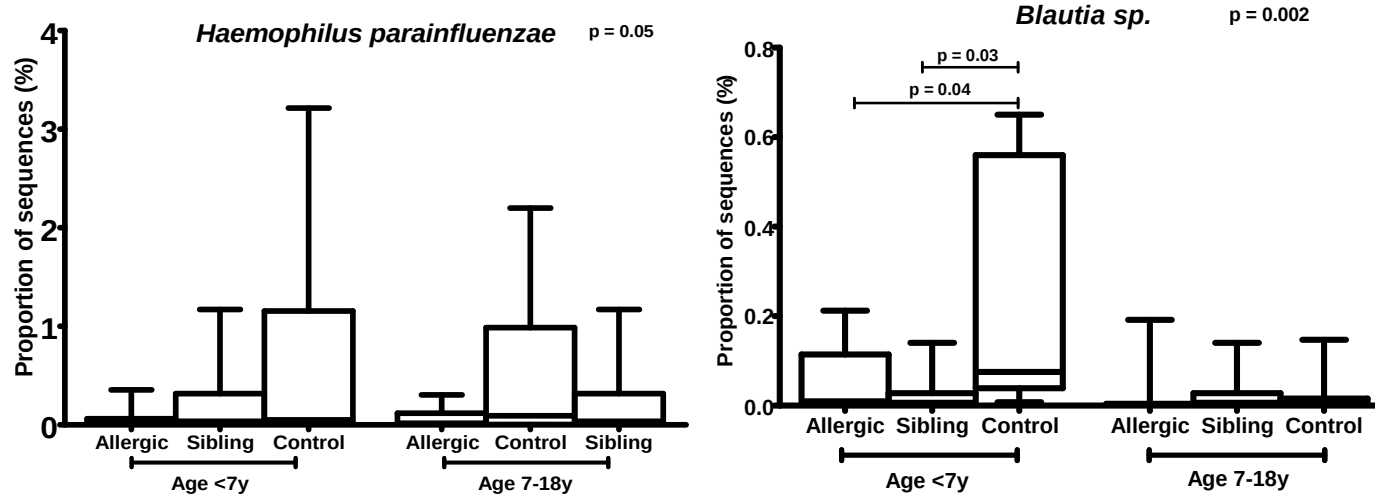
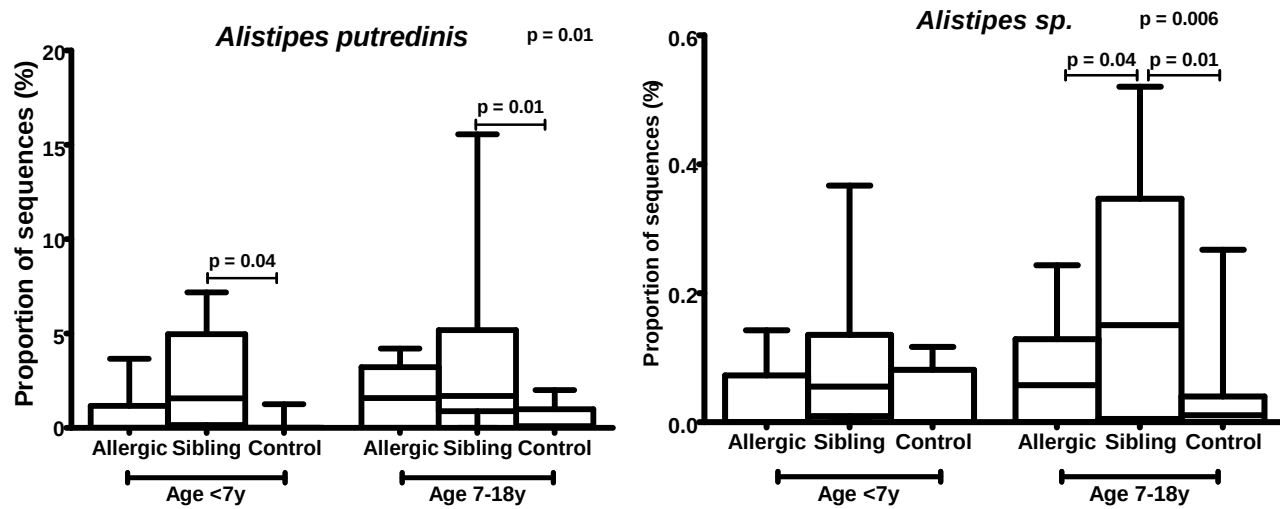


Figure 3. Supplemental



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