

Infant gut bacterial community composition and food-related manifestation of atopy in early childhood

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Abstract

Background

Immunoglobulin E-mediated food allergy (IgE-FA) has emerged as a global public health concern. Immune dysregulation is an underlying mechanism for IgE-FA, caused by “dysbiosis” of the early intestinal microbiota. We investigated the association between infant gut bacterial composition and food-related atopy at age 3–5 years using a well-characterized birth cohort.

Methods

The study definition of IgE-FA to egg, milk, or peanut was based on physician panel retrospective review of clinical and questionnaire data collected from birth through age 3–5 years. Using 16S rRNA sequencing, we profiled the bacterial gut microbiota present in stool specimens collected at 1 and 6 months of age.

Results

Of 447 infants with data for analysis, 44 (9.8%) met physician panel review criteria for IgE-FA to ≥ 1 of the three allergens. Among children classified as IgE-FA at 3–5 years, infant stool samples showed significantly less diversity of the gut microbiota compared with the samples of children classified as no IgE-FA at age 3–5 years, especially for milk and peanut (all covariate-adjusted p 's for alpha metrics $<.007$). Testing of individual operational taxonomic units (OTUs) revealed 6-month deficiencies in 31 OTUs for IgE-FA

compared with no IgE-FA, mostly in the orders *Lactobacillales*, *Bacteroidales*, and *Clostridiales*.

Conclusions

Variations in gut microbial composition in infant stool were associated with a study definition of IgE-FA at 3–5 years of age. This included evidence of a lack of bacterial diversity, deficiencies in specific OTUs, and delayed microbial maturation. Results support dysbiosis in IgE-FA pathogenesis.

Abbreviations

BIC

Bayesian information criteria

BSA

Bovine serum albumin

CTAB

Cetyltrimethylammonium bromide

FDR

False discovery rate

HFHS

Henry Ford Health System

IgE

Immunoglobulin E

IgE-FA

Immunoglobulin E-mediated food allergy

MAAP

Microbes, allergy, asthma, and pets

OUT

Operational taxonomic unit

SPT

Skin prick test

WHEELS

Wayne County Health Environment Allergy and Asthma Longitudinal Study

Key Message

Compositional differences in gut bacterial colonization were observed for children who developed IgE-mediated food allergy compared with those that did not. Results support a theory of elevated risk due to dysbiosis and delays in microbial maturation. The importance of further research in this area lies in the potential for developing interventions that can prevent development of IgE-FA.

1 INTRODUCTION

The current 8% prevalence of food allergy for US children represents a 50% increase between 1997 and 2011.^{1,2} Food allergy can be life-threatening and undermines the quality of life of affected children and their families.^{1,2}

Food allergy is a reproducible inflammatory response induced by immunoglobulin E activation of mast cells and basophils upon exposure to a given food.³ Immunoglobulin E (IgE)–mediated food allergy (IgE-FA) occurring during infancy can be preceded by atopic eczema and increases the risk of rhinitis and asthma in later childhood.³ Immune dysregulation is an important contributor to IgE-FA.⁴ In a healthy state, oral exposure to innocuous antigens (eg, food proteins) leads to interactions with specific antigen-presenting cells followed by the induction of T regulatory cell suppression of immune responses, or oral tolerance.³ In IgE-FA, exposure to common food proteins results in an inappropriate T helper 2 cell–mediated response to epitopes of the offending food.³ Examining associations along the causal pathway with IgE-FA could inform processes involved in early childhood allergic disease.

Immune processes leading to the induction or failure of oral tolerance are actively influenced by the gut microbiome.⁵ In allergy-free individuals, commensal bacteria stimulate responses leading to immune tolerance of innocuous allergens.⁵ Aberrant colonization of bacteria in the gut (dysbiosis) increases susceptibility to atopy.⁵ Delayed colonization of gut bacteria leads to irregularities in the development of gut-associated lymphoid tissues, impacting downstream immune pathways.⁶ Associations between gut microbiota composition and food sensitization or IgE-FA are reported in murine and human studies.^{7,8}

Well-characterized birth cohorts are suited to exploring how gut bacterial colonization impacts IgE-FA risk. We use data from the Microbes, Allergy, Asthma, and Pets (MAAP) Research Program, drawn from the Wayne County Health Environment Allergy and Asthma Longitudinal Study (WHEALS) birth cohort⁹ to explore early gut bacterial composition and risk of IgE-FA to egg, milk, or peanut at age 3–5 years.⁹

2 METHODS

2.1 Study population

The Institutional Review Board at Henry Ford Health System (HFHS) approved this research. The WHEALS birth cohort was established to identify environmental factors related to the development of allergy and asthma.⁹ Methods, eligibility, and recruitment have been described previously.⁹ Briefly, pregnant women aged 21–45 years, residing in metropolitan Detroit and receiving prenatal care at selected HFHS obstetric clinics from September 2003 through November 2007, were recruited. Infant blood samples were collected at 6- and 12 months home visits. At a 24 months clinic visit, infant blood was collected to measure allergen-specific serum IgE as described previously,⁹ and skin prick tests (SPTs) were

conducted using a Duotip-test device (Lincoln Diagnostics Inc.), and including, but not limited to, egg, milk, and peanut. A wheal diameter ≥ 3 mm larger than saline control was considered positive for SPTs. Infant stool samples collected at 1- and 6 months home visits were transported to the laboratory in cryovials, stored at -80°C , and shipped to UCSF, where they underwent sequencing of the V4 region of the 16S rRNA gene using the Illumina NextSeq (Appendix 1).¹⁰ Parents were interviewed at infant ages 1, 6, 12, and 24 months for medical history, and at ages 3–5 years for infant food avoidance, gastrointestinal symptoms, and reactions to food. Infant medical records (requested for those outside of HFHS) were reviewed from birth through age 5 years. The definition of IgE-FA used in this study has also been described previously.¹¹ Briefly, a panel of two board-certified allergists reviewed clinical and interview data from birth through age 3–5 years to classify infants as highly likely, likely, or unlikely to have IgE-FA. This was a two-step process. In Step 1, we identified infants with at least two of the following three characteristics for egg, milk, or peanut: (1) ≥ 1 specific IgE level ≥ 0.35 IU/ml, (2) a positive SPT result, or (3) parental report of infant symptoms potentially related to food allergy plus ≥ 1 specific IgE level ≥ 0.10 IU/ml. Infants that did not exhibit two of these three characteristics were automatically classified as “unlikely” to have IgE-FA. Infants with at least two of the three characteristics were forwarded to the physician panel for classification (Step 2) using study protocols based on recently published guidelines.^{12, 13} A third allergist ruled on discordant decisions. For this analysis, “highly likely” and “likely” were collapsed into a single category and compared with “unlikely.” Report of symptoms was an integral part of the classification. The classification was also heavily influenced by IgE and/or SPT results meeting the 95% predictive decision points,¹³ and medical chart documentation of a physician diagnosis of IgE-FA or results of an oral food challenge.

2.2 Statistical analysis

Main effects and interaction effects were considered significant at $p < .05$ and $p < .10$, respectively. Characteristics of children included and excluded from the analysis were compared using ANOVA and the chi-squared test for numeric and categorical covariates, respectively. Children with and without IgE-FA were compared using Kruskal-Wallis for numeric covariates and Fisher's exact test for categorical covariates. Alpha diversity measures of bacterial richness, Pielou's evenness, Faith's phylogenetic diversity (PD), and Shannon's diversity were estimated using QIIME¹⁴ and the R vegan package.¹⁵ Measurements used exact age at sample collection and were fit using generalized estimating equations (GEE) with a Gaussian link to account for within-subject correlations. Differences in alpha diversity by IgE-FA were examined using time interactions, followed by main effects if interactions were nonsignificant. Composition of the gut microbiota was defined using unweighted and weighted UniFrac (phylogenetic),¹⁶ as well as Canberra and Bray-Curtis dissimilarity (non-phylogenetic). Compositional differences were assessed using PERMANOVA in the R package vegan.¹⁵ Individual operational taxonomic unit (OTU) tests were performed using zero-inflated negative binomial models (or standard negative

binomial models if convergence failed), with **Benjamini-Hochberg** false discovery rate (FDR)–adjusted p-values¹⁷ computed to account for multiple testing (FDR-adjusted $p < .05$, significant). OTUs were only tested if they were detected in $\geq 25\%$ of samples. Bacterial microbiota-for-age z-scores (BMAZ) were calculated to determine whether microbial maturity (given fixed chronological age) differed in children with IgE-FA. Random forest models were fit using the randomForest package, with actual age at stool sample collection as the outcome, and the relative abundance of OTUs as predictive features. To identify a small subset of taxa that explained a large portion of the variability in age, the rfcv function of the randomForest package was applied, using a fivefold cross-validation.¹⁸ This sparse model was then used to predict age at stool sample collection; BMAZ was calculated as described in Subramanian et al, 2014,¹⁹ with each month used as an age category, except for 2–4 and 8–10 months, which were collapsed due to data sparsity. Differences in BMAZ by IgE-FA were tested using GEE models, as described previously. The GEEmediate R package was utilized to test the mediating effect of eczema by age 2 for all microbiota metrics (alpha diversity, beta diversity, and BMAZ).²⁰ For beta diversity, the first principal coordinate of each metric was used in the mediation models.

3 RESULTS

Of the 590 children with sufficient data for IgE-FA classification, 447 had stool samples for microbiota analysis ($n = 44$ with IgE-FA and $n = 403$ with no IgE-FA). Of the 447 children, 156 had a stool sample at 1 month only, 118 had a stool sample at 6 months only, and 173 had a stool sample for both time points. Among the 44 children with IgE-FA, 59% were allergic to one food, 30% were allergic to two, and 11% were allergic to all three foods. The most common IgE-FA in our sample was egg (73% of the IgE-FA children), followed by peanut (59%) and milk (20%).

Compared with those excluded from analysis, mothers of infants in the analytic sample were older ($p = .020$), reported more education ($p < .001$), higher household income ($p < .001$), a history of atopy ($p = .014$), and greater likelihood of exclusive breastfeeding ($p = .026$), and were less likely to be urban residents ($p = .014$) and/or exposed to environmental tobacco smoke ($p = .007$) (Table S1). Children in the analytic sample meeting study definition of IgE-FA (Table 1) were more likely to have diagnosed eczema by age 2 (37.2% vs. 19.7%, $p = .017$) compared with non-IgE-FA.

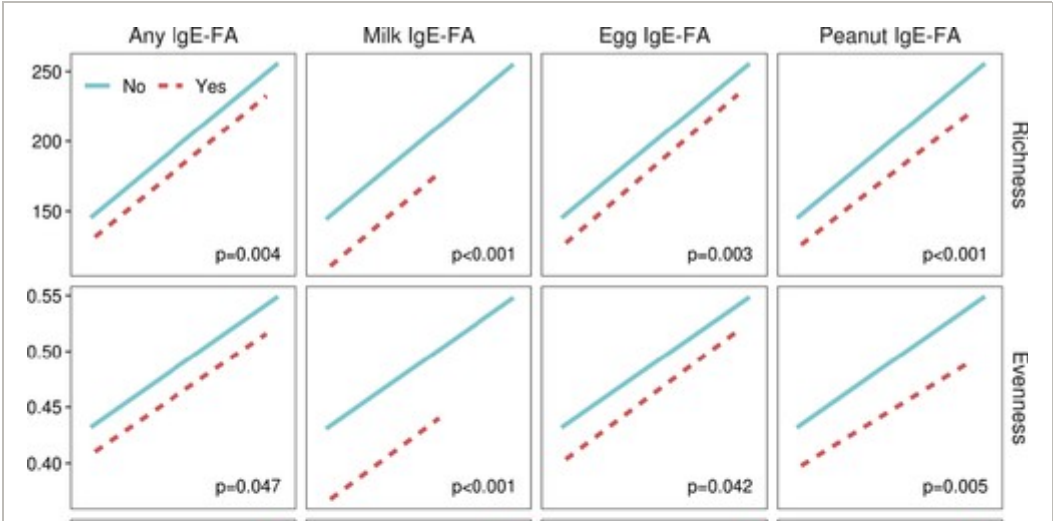
TABLE 1. Selected cohort characteristics for food-allergic and non–food-allergic children included in the analyses ($n = 447$)

Covariate	IgE-FA $n = 44$	No IgE-FA $n = 403$	p -value ¹
Child sex			
Male	23 (52.3)	215 (53.3)	1.000

Covariate	IgE-FA <i>n</i> = 44	No IgE-FA <i>n</i> = 403	<i>p</i> -value ¹
Female	21 (47.8)	188 (46.7)	.071
Child race			
African American	30 (68.2)	239 (59.3)	
White	6 (13.6)	100 (24.8)	
Hispanic/Latino	1 (2.3)	27 (6.7)	
Other	7 (15.9)	37 (9.2)	.460
Household income			
<\$40K	12 (27.3)	121 (30.0)	
\$40K - <\$80K	8 (18.2)	107 (26.6)	
\$80K - >=\$100K	18 (40.9)	122 (30.2)	
Refused	6 (13.6)	53 (13.2)	.875
Urban residence	22 (50.0)	207 (51.4)	

¹ Kruskal-Wallis test for numerical covariates and Fisher's exact test for categorical covariates.

When all stool samples were modeled, all alpha diversity metrics were lower in children meeting study criteria for IgE-FA compared to those without, after covariate adjustment (Figure 1, Table 2), and this effect did not significantly differ over time (Table 2, all interaction $p \geq .13$). Effects were largest for milk-allergic children compared with non–milk-allergic children, followed by peanut and egg. Results were similar using specific IgE ≥ 0.35 IU/ml (sensitization) as an outcome (Table 2), but a significant effect was observed only for peanut (Table 2).



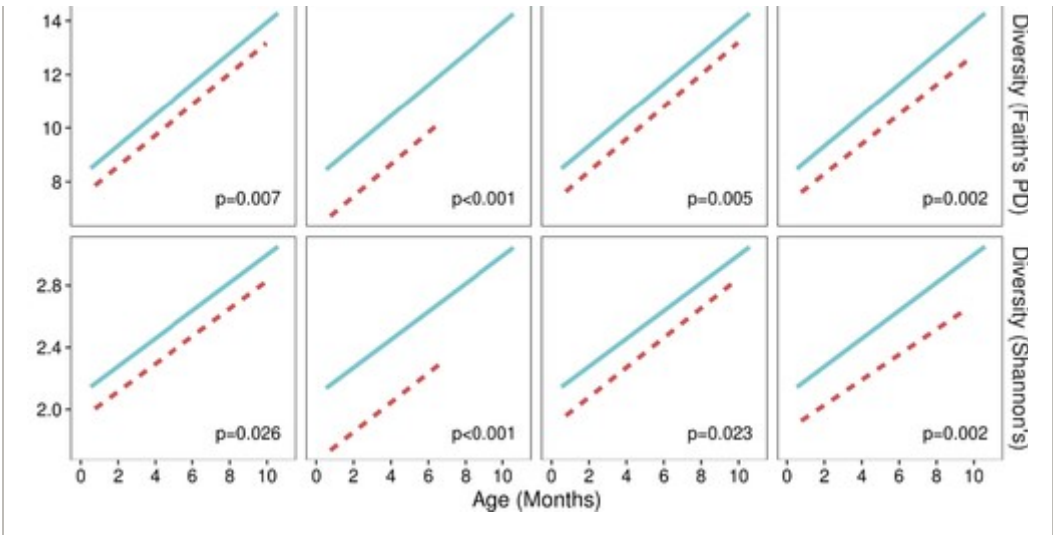


FIGURE 1

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Difference in bacterial alpha diversity metrics by study definition of IgE-mediated food allergy (IgE-FA), after adjusting for exact age at stool sample collection, child race, maternal history of allergies or asthma, and breastfeeding status at 1 month

TABLE 2. Difference in bacterial alpha diversity metrics by study definition of IgE-mediated food allergy (IgE-FA) and food sensitization ^{1,2}, ^{1,2}

Metric	Any IgE-FA			Milk IgE-FA			Egg IgE-FA			Peanut IgE-FA	
	Interx ³ <i>p</i>	β (95% CI) ⁴	<i>p</i>	Interx ³ <i>p</i>	β (95% CI) ⁴	<i>p</i>	Interx ³ <i>p</i>	β (95% CI) ⁴	<i>p</i>	Interx ³ <i>p</i>	
Richness	0.307	-17.39 (-29.31, -5.47)	.004	0.265	-33.3 (-49.84, -16.76)	<.001	0.493	-20.15 (-33.66, -6.65)	.003	0.179	
Evenness	0.581	-0.025 (-0.05, 0)	.047	0.224	-0.06 (-0.09, -0.03)	<.001	0.395	-0.029 (-0.057, -0.001)	.042	0.352	
Faith's PD ⁵	0.697	-0.84 (-1.45, -0.23)	.007	0.132	-1.76 (-2.67, -0.85)	<.001	0.92	-1 (-1.7, -0.3)	.005	0.274	
Shannon's ⁶	0.777	-0.17 (-0.32, -0.02)	.026	0.343	-0.39 (-0.55, -0.23)	<.001	0.549	-0.2 (-0.36, -0.03)	.023	0.546	
	Any Food Sensitization			Milk Sensitized			Egg Sensitized			Peanut Se	

Metric	Any IgE-FA			Milk IgE-FA			Egg IgE-FA			Peanut IgE-FA	
	Interx <i>p</i> ³	β (95% CI) ⁴	<i>p</i>	Interx <i>p</i> ³	β (95% CI) ⁴	<i>p</i>	Interx <i>p</i> ³	β (95% CI) ⁴	<i>p</i>	Interx <i>p</i> ³	

¹ Table 2 analyses based on both 1- and 6 months samples.

² Sensitization based on serum specific IgE ≥0.35 IU/ml for egg, milk, or peanut.

³ Interaction *p*-value; tests if the association between alpha diversity and outcome is time-dependent, after adjusting for exact age at stool sample collection, child race, maternal history of allergies or asthma, and breastfeeding status at 1 month.

⁴ Interpreted as the mean difference in alpha diversity across time, comparing IgE-FA with non-IgE-FA, and sensitized with non-sensitized, after adjusting for exact age at stool sample collection, child race, maternal history of allergies or asthma, and breastfeeding status at 1 month.

⁵ Faith's phylogenetic diversity.

⁶ Shannon's diversity index.

Bacterial compositional differences by IgE-FA were tested using PERMANOVA (Table 3). Adjusting for covariates, significant compositional differences by IgE-FA were present only at 6 months of age (Table 3). Only unweighted UniFrac and Canberra distances revealed significant differences at 6 months for all IgE-FA outcomes. Sensitization to “any food” and to peanut was associated with 1 months community composition, only.

TABLE 3. Association between early life microbiome composition and study definition of IgE-mediated food allergy (IgE-FA) and food sensitization¹

Outcome	Metric	1 Month ²			6 Months ²		
		<i>N</i>	<i>p</i> -value	<i>R</i> ²	<i>N</i>	<i>p</i> -value	<i>R</i> ²
Any IgE-FA	Unweighted UniFrac	323	.108	0.004	282	.013	0.006
	Weighted UniFrac	323	.674	0.002	282	.009	0.012
	Canberra	323	.196	0.003	282	.008	0.005
	Bray-Curtis	323	.285	0.003	282	.018	0.006
Milk IgE-FA	Unweighted UniFrac	323	.148	0.004	282	.008	0.006
	Weighted UniFrac	323	.313	0.004	282	.008	0.014
	Canberra	323	.075	0.004	282	.008	0.005
	Bray-Curtis	323	.145	0.004	282	.071	0.005
Egg IgE-FA	Unweighted UniFrac	323	.089	0.004	282	.032	0.005

Outcome	Metric	1 Month ²			6 Months ²		
		N	p-value	R ²	N	p-value	R ²
	Weighted UniFrac	323	.603	0.002	282	.040	0.009
	Canberra	323	.123	0.003	282	.020	0.004
	Brav-Curtis	323	.370	0.003	282	.021	0.006

¹ Serum specific IgE ≥0.35 IU/ml for egg, milk, or peanut.

² After adjusting for exact age at stool sample collection, child race, maternal history of allergies or asthma, and breastfeeding status at 1 month.

IgE-FA was significantly associated with the abundance of 8 OTUs (Figure 2) after covariate adjustment, 5 of which were in lower abundance in IgE-FA children. At 6 months of age, 20 of 31 significant OTUs identified were deficient for those with IgE-FA, primarily *Bacteroidales* and *Clostridiales*. OTUs at 6 months overabundant in IgE-FA children were mostly of the order *Bifidobacteriales*.

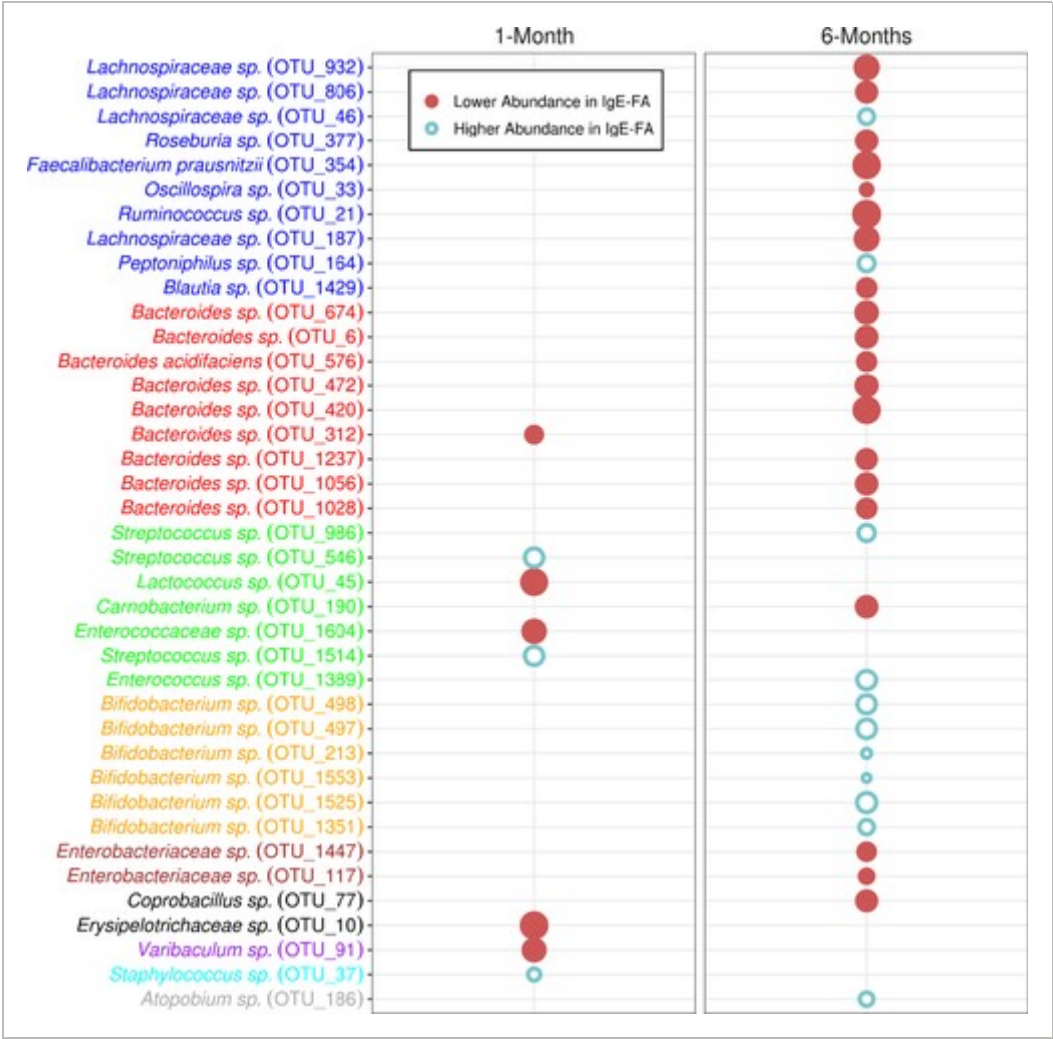


FIGURE 2

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OTUs at 1 and 6 months of age significantly associated with study definition of IgE-FA ($p_{FDR} < 0.05$), after adjusting for exact age at stool sample collection, child race, maternal history of allergies or asthma, and breastfeeding status at 1 month. OTUs are colored by taxonomic order. Color of points represent direction of association, **while size of points represents effect size as determined by ZINB/NB models**. OTUs are grouped and colored by taxonomic order. From top: blue = Clostridiales, red = Bacteroidales, green = Lactobacillales, orange = Bifidobacteriales, brown = Enterobacteriales, black = Erysipelotrichales, purple = Actinomycetales, cyan = Bacillales, dark gray = Coriobacteriales

Children with IgE-FA had significantly lower adjusted bacterial microbiota-for-age z-scores (BMAZ) compared with non-IgE-FA children (Figure 3; β (95% CI) = -0.70 ($-1.08, -0.33$), $p < .001$). This effect did not significantly differ across time (interaction $p = .97$). No mediating effect was observed for the association between alpha diversity, beta diversity, and BMAZ to study definition of IgE-FA ($p \geq .44$) (Table S2).

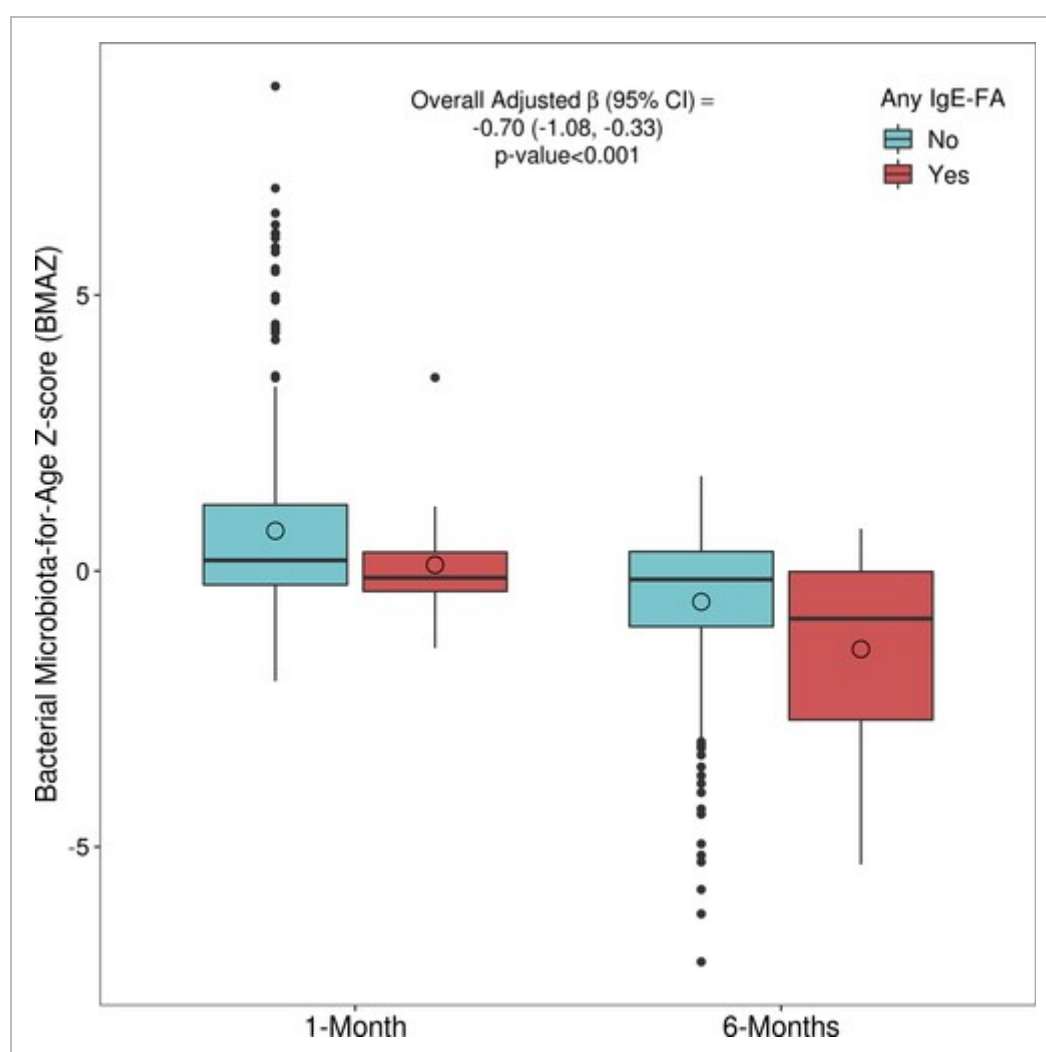


FIGURE 3

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Bacterial microbiota-for-age z-score (BMAZ) by study definition of “any IgE-FA”. Effect estimate and p-value are for the overall effect, after adjusting for exact age at stool sample collection, child race, maternal history of allergies or asthma, and breastfeeding status at 1 month. The effect was not significantly different across time (interaction p -value = 0.97)

4 DISCUSSION

We observed deficiencies in the alpha diversity of gut microbiota for birth cohort infants meeting study criteria for IgE-FA by age 3–5 years, primarily at 6 months of age, based on dissimilarity measures. We observed an overabundance of several *Bifidobacteriales* OTUs and a deficiency in several *Bacteroidales* and *Clostridiales* OTUs in IgE-FA children at age 6 months. Microbiota-for-age z-scores suggest delayed maturity in the infant gut microbiota of children with IgE-FA. Suggested differences in rare taxa or taxa of low abundance (UniFrac, Canberra) need further exploration. Results suggest dysbiosis is related to oral tolerance and IgE-FA.

Microbial composition can modify the risk of IgE-FA through innate and adaptive immunity.⁴ Unwanted microbial antigens bound by secretory IgA transferred maternally from breastfeeding are handled by the innate immune system.⁴ Oligosaccharides in human milk that induce growth of *Bifidobacterium* and *Lactobacillus* in the infant gut also induce production of IL-10 and IgA.⁴ *Clostridia*, particularly clusters IV and XIVa, activate the release of TGF- β . IgA and cytokines IL-10 and TGF- β are inducers of T regulatory cells that suppress undesirable immune reactions. Gut bacteria also participate in the fermentation of complex carbohydrates generating short-chain fatty acids, which contribute to the regulation of inflammatory responses by influencing B-cell function and intestinal barrier function.^{4, 5, 21} Thompson-Chagoyan [2011] showed that changes in infant gut composition were concomitant with decreases in levels of specific IgE against cow's milk antigens after providing hydrolyzed formula to infants with cow's milk allergy.²² These infants had higher concentrations of butyric acid than their non-allergic counterparts²² providing evidence that deficiencies in taxa may represent reduced capacity to offset inflammatory processes.

Previous studies have looked at gut bacterial colonization and risk of food sensitization and IgE-FA with reports of a potential “signature” for food sensitization,²³ or results suggestive of delays in gut maturity.^{24, 25} In VDAART, investigators reported deficiencies in *Clostridium* that were significant for both food sensitization and food allergy,²⁶ but, unlike our analysis, showed subgroup variations for race, mode of delivery, and age at introduction of solid food.²⁶ Similarly, in the Consortium of Food Allergy (CoFAR) observational study of milk allergy, *Clostridia* and *Firmicutes* were enriched in the guts of children whose milk allergy resolved by 8 years.⁸ The CoFAR study provided additional evidence of decreased fatty acid metabolism, emphasizing potential mechanisms by which risk of IgE-FA is modified.⁸

Our findings contrast with those of studies showing higher abundance of *Bifidobacterium* associated with reduced risk of atopic disease.^{27, 28} Our results are more aligned with those

reported by Stokholm et al., using the Copenhagen Prospective Studies on Asthma in Childhood (COPSAC), in which gut colonization at 1 year for infants who developed asthma at age 5 years resembled that of healthy infants at 1 month, indicating delayed gut maturation.²⁵ Since *Bifidobacterium* is 40%–80% of gut colonized bacteria shortly after birth, our results may support the hypothesis that delayed gut maturity influences IgE-FA risk.²⁵

We did not find that atopic eczema, which typically precedes IgE-FA,³ mediates the relationship between microbiota composition and IgE-FA in our study. A systematic review (2019) found that 5 of 11 observational studies on this topic reported lower gut diversity associated with eczema.²⁸ While no specific bacterial species have emerged in the literature consistently, *Bifidobacterium* appears in several reports.²⁷⁻²⁹ Zhang, et al. reported decreased abundance of *Bifidobacterium* associated with eczema only for infants >6 months.²⁷ Ismail et al. found modulation of eczema risk (among children at high risk of atopy) driven by certain *Bifidobacterium* species²⁸; however, Ta, et al. found atopic eczema was associated with delayed colonization of *Bacteroides*, but not *Bifidobacterium*.²⁹ Evidence of immune modulatory effects that are specific to certain species highlights the need for further research.

Study variation in the orders, families, and genera of the taxa reportedly associated with sensitization or IgE-FA could be due to a myriad of factors including differences in study outcomes, age at sample collection, specific allergen under study, characteristics of the study samples, or dissimilarities in sample storage, processing, and analysis.¹² The implications of individual-level variation in the characteristics of infant intestinal microbiota continue to emerge. The substantial variation in gut colonization of healthy individuals suggests that a defined composition of microbial taxa universally present is unlikely.³⁰ A “healthy” gut is more likely defined by a “core” set of functions performed by a variety of colonized bacteria, as opposed to the presence of a fixed set of taxa.³⁰

We did not conduct oral food challenges in this birth cohort; however, documentation of an oral food challenge in the medical chart was a chief consideration in our study definition of IgE-FA.¹¹ We classified children from birth to 5 years as having IgE-FA using clinical and symptom data that may have been acquired at different time points, and we do not use exact age at diagnosis in our analysis. Misclassification is possible if clinical evidence of IgE-FA at 2 years resolved by age 3–5. Due to small sample size, we did not assess the impact of antibiotic exposure (reported by only 3% of cohort infants), nor the impact of environmental and sociocultural factors on the relationship between infant gut bacterial composition and IgE-FA, as done in an earlier publication.⁹ We acknowledge that our observed associations are not causal.

Despite limitations, our analysis supports modulation of IgE-FA risk by colonization of infant gut bacteria. Bacterial colonization is a potentially modifiable factor along the causal pathway to IgE-FA. Continued research in this area creates potential for intervention and prevention of IgE-FA in infants and children.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTION

Christine LM Joseph: Conceptualization (lead); Formal analysis (supporting); Writing – review & editing (lead). **Alexandra R Sitarik:** Data curation (lead); Formal analysis (lead); Visualization (equal); Writing – review & editing (supporting). **Haejin Kim:** Formal analysis (equal); Writing – review & editing (equal). **Edward Zoratti:** Conceptualization (equal); Writing – review & editing (equal). **Gary Huffnagle:** Formal analysis (equal); Writing – review & editing (equal). **Kei E Fujimura:** Investigation (supporting); Writing – review & editing (equal). **Germaine Jia Min Yong:** Investigation (supporting); Writing – review & editing (equal). **Albert M Levin:** Formal analysis (equal); Validation (equal); Writing – review & editing (equal). **Susan Lynch:** Formal analysis (equal); Investigation (equal); Supervision (lead); Writing – review & editing (equal). **Dennis R. Ownby:** Conceptualization (equal); Writing – review & editing (equal). **Nicholas W Lukacs:** Conceptualization (equal); Writing – review & editing (equal). **Brent Davidson:** Methodology (equal); Project administration (equal); Writing – review & editing (equal). **Charles Barone:** Methodology (equal); Project administration (equal); Writing – review & editing (equal). **Christine Cole Johnson:** Data curation (equal); Formal analysis (equal); Methodology (equal); Supervision (equal); Validation (equal); Writing – review & editing (equal).

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REFERENCES



1

Gupta RS, Warren CM, Smith BM, et al. The public health impact of parent-reported childhood food allergies in the United States. *Pediatrics*. 2018; **142**(6):e20181235.
[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

2

Gupta RS, Springston EE, Warrierr MR, et al. The prevalence, severity, and distribution of childhood food allergy in the United States. *Pediatrics*. 2011; **128**: e9- e17.
[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

3

Sicherer SH, Sampson HA. Food allergy: a review and update on epidemiology parthenogenesis, diagnosis, prevention, and management. *J Allergy Clin Immunol*. 2017; **141**: 41- 58.
[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

4

Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. *Nature*. 2012; **489**: 231- 241.
[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

5

Yu W, Freeland DMH, Nadeau KC. Food allergy: immune mechanisms, diagnosis and immunotherapy. *Nat Rev Immunol*. 2016; **16**: 751.
[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

6

Ling Z, Li Z, Liu X, et al. Altered fecal microbiota composition associated with food allergy in infants. *Appl Environ Microbiol*. 2014; **80**: 2546- 2554.
[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

7

Feehley T, Plunkett CH, Bao R, et al. Healthy infants harbor intestinal bacteria that protect against food allergy. *Nat Med*. 2019; **25**: 448- 453.
[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

8 Bunyavanich S, Shen N, Grishin A, et al. Early-life gut microbiome composition and milk allergy resolution. *Allergy Clin Immunol*. 2016; **138**: 1122- 1130.

[Crossref](#) | [CAS](#) | [Google Scholar](#)

9 Levin AM, Sitarik AR, Havstad SL, et al. Joint effects of pregnancy, sociocultural, and environmental factors on early life gut microbiome structure and diversity. *Sci Rep*. 2016; **6**: 31775.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

10 Sitarik AR, Arora M, Austin C, et al. Fetal and early postnatal lead exposure measured in teeth associates with infant gut microbiota. *Environ Int*. 2020; **144**: 106062.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

11 Ezell JM, Ownby DR, Zoratti EM, et al. Using a physician panel to estimate food allergy prevalence in a longitudinal birth cohort. *Ann Epidemiol*. 2014; **24**: 551- 553.

[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

12 Boyce JA, Assa'ad A, Burks AW, et al. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-sponsored expert panel report. *J Allergy Clin Immunol*. 2010; **126**: 1105- 1118.

[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

13 Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol*. 2001; **107**: 891- 896.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

14 Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods*. 2010; **7**: 335- 336.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

15 Oksanen J, Blanchet G, Friendly M, et al. vegan: Community ecology package. *R Package Version*. 2016; **2**(4-4): 2017.

[Google Scholar](#)

16 Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl Environ Microbiol*. 2005; **71**: 8228- 8235.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

17 Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc. Series B (Methodological)*. 1995; 289- 300.

[Google Scholar](#)

18 Liaw A, Wiener M. Classification and regression by randomForest. *R News*. 2002; **2**: 18- 22.

[Google Scholar](#)

19 Subramanian S, Huq S, Yatsunenko T, et al. Persistent gut microbiota immaturity in malnourished Bangladeshi children. *Nature*. 2014; **510**: 417- 421.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

20 *GEEmediate: Mediation Analysis for Generalized Linear Models Using the Difference Method*. R package version 1.1.3. Accessed 2019. [https://www.GEEmediate.pdf\(r-project.org\)](https://www.GEEmediate.pdf(r-project.org))

[Google Scholar](#)

21 Wang RX, Lee JS, Campbell EL, Colgan SP. Microbiota-derived butyrate dynamically regulates intestinal homeostasis through regulation of actin-associated protein synaptopodin. *Proc Natl Acad Sci*. 2020; **117**: 11648- 11657.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

22 Thompson-Chagoyan OC, Fallani M, Maldonado J, et al. Faecal microbiota and short-chain fatty acid levels in faeces from infants with Cow's milk protein allergy. *Int Arch Allergy Immunol*. 2011; **156**: 325- 332.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

23 Chen CC, Chen KJ, Kong MS, Chang HJ, Huang JL. Alterations in the gut microbiota of children with food sensitization in early life. *Pediatr Allergy Immunol*. 2016; **27**: 254- 262.

[Wiley Online Library](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

24 Azad MB, Konya T, Guttman DS, et al. Infant gut microbiota and food sensitization: associations in the first year of life. *Clin Exp Allergy*. 2015; **45**: 632- 643.

[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

25 Stokholm J, Blaser MJ, Thorsen J, et al. Maturation of the gut microbiome and risk of asthma in childhood. *Nat Commun*. 2018; **9**: 141.

[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

26 Savage JH, Lee-Sarwar KA, Sordillo J, et al. A prospective microbiome-wide association study of food sensitization and food allergy in early childhood. *Allergy*. 2018; **73**: 145- 152.

[Wiley Online Library](#) | [CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

27 Zhang Y, Jin S, Wang J, et al. Variations in early gut microbiome are associated with childhood eczema. *FEMS Microbiol Lett*. 2019; **366**: fnz020.

[Crossref](#) | [CAS](#) | [PubMed](#) | [Google Scholar](#)

28 Petersen E, Skov L, Thyssen JP, Jensen P. Role of the gut microbiota in atopic dermatitis: a systematic review. *Acta Derm Venereol*. 2019; **99**: 5- 11.

[CAS](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

29 Ta LDH, Chan JCY, Yap GC, et al. A compromised developmental trajectory of the infant gut microbiome and metabolome in atopic eczema. *Gut Microbes*. 2020; **12**: 1801964.

[Crossref](#) | [Google Scholar](#)

30 Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. *Genome Med.* 2016; 8: 1- 11.

[Crossref](#) | [PubMed](#) | [Web of Science®](#) | [Google Scholar](#)

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Supplement, Appendix 1

Infant stool was collected by field staff during home visits targeting 1 and/or 6 months of age. Stool was placed into cryovials, transported to the laboratory and stored at -80°C . DNA was extracted from stool samples using a modified cetyltrimethylammonium bromide (CTAB) buffer-based protocol¹. Briefly, 500ul modified CTAB extraction buffer was added to 25 mg of stool in a 2 ml Lysing Matrix E tube (MP Biomedicals, Santa Ana, CA) prior to incubation at 65°C for 15 min. Samples were bead-beaten (5.5 m s^{-1} , 30 sec) in a Fastprep-24 (MP Biomedicals, Santa Ana, CA) and then centrifuged ($16000 \times g$, 5 min) before the top aqueous phase was transferred to a 2ml polypropylene 96-well plate (USA Scientific, Ocala, FL). A further 500ul modified CTAB extraction buffer was added to each LME tube, which was similarly bead-beaten and centrifuged to collect a total of 1ml aqueous phase per sample. After adding 1ml of phenol:chloroform:isoamyl alcohol (25:24:1) to the collected aqueous supernatant, samples were centrifuged ($3200 \times g$, 20 min, 4°C) and the resulting top aqueous phase was transferred to a new 2ml polypropylene 96-well plate (USA Scientific, Ocala, FL). PEG/NaCl (2v/v) was added to the collected aqueous supernatant and incubated at room temperature for 2 h. Samples were then centrifuged ($3200 \times g$, 60 min, 4°C), washed with ice cold 70% EtOH and resuspended in 30 μl TE buffer (Invitrogen, Calsbad, CA).

The amplification of the V4 region of the 16S rRNA gene was performed in 25- μL reactions using 0.025 U Takara Hot Start ExTaq (Takara Mirus Bio Inc., Madison, WI), 1X Takara buffer with MgCl_2 , 0.4pmol/ μL of F515 and R806primers, 0.56mg/mL of bovine serum albumin (Roche Applied Science, Indianapolis, IN), 200 μM of dNTPs and 10 ng of genomic DNA. Reactions were performed in triplicate with the following: initial denaturation (98°C , 2min), 30 cycles of 98°C (20 sec), annealing at 50°C (30 sec), extension at 72°C (45sec), and final extension at 72°C (10 min). Amplicons were verified using a 2% Tris/Borate/EDTA agarose e-gel (Life Technologies, Grand Island, NY), cleaned and normalized using SequalPrep Normalization Plates (Applied Biosystems, Foster City, CA), and further quantified using the Qubit 2.0 Fluorometer and the double-stranded DNA HS Assay Kit (Life Technologies). Samples were pooled in equal moles at concentrations of 5 ng, purified using AMPure SPRI beads (Beckman Coulter, Brea, CA), denatured and diluted to 2 nM, and 5 pM was loaded onto the Illumina Nextseq cartridge with 40% (v/v) of denatured 12.5 pM PhiX spike-in control.

Bacterial paired-end sequences were assembled using FLASH v1.2.7² requiring a minimum base pair overlap of 25bp and demultiplexed by barcode using QIIME v1.8³. Quality filtering was performed using USEARCH v8.0.1623⁴ to remove reads with >2 expected errors. Quality reads were dereplicated at 100% sequence identity, clustered at 97% sequence identity into operational taxonomic units (OTUs), filtered of chimeric sequences by UCHIME⁵, and mapped back to resulting OTUs using UPARSE⁶; sequence reads that failed to cluster with a reference sequence were clustered *de novo*. Taxonomy was assigned to the OTUs using the Greengenes database v13_5⁷. Sequences were aligned using PyNAST⁸, and FastTree 2.1.3⁹ was used to build a phylogenetic tree. A rarefaction depth of 60,000 reads per sample was selected by using rarefaction curves, based on the plateau of reads and maximizing sample inclusion. Rather than selecting a single rarefaction table, we multiply rarefied the data in order to ensure a representative community was selected for each sample¹⁰. Briefly, multiple rarefied OTU tables are calculated, and for each sample, the distance between the sample-specific rarefied vectors calculated. The rarefied vector that is the minimum average distance from itself to all other rarefied vectors is selected, as it is considered the most representative for that sample. A total of 580 children had at least 1 stool sample in the final rarefied OTU table. Of 447 infants with data for analysis, the 1-month stool samples were collected between 16 and 138 days of age

(mean=40, SD=19), while 6-month stool samples were collected between 168 and 322 days of age (mean=207, SD=32).

References for Supplement, Appendix 1

1. DeAngelis KM, Brodie EL, DeSantis TZ, Andersen GL, Lindow SE, Firestone MK. Selective progressive response of soil microbial community to wild oat roots. *The ISME journal* 2009; 3:168-78.
2. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 2011; 27:2957-63.
3. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010; 7:335-6.
4. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010; 26:2460-1.
5. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011; 27:2194-200.
6. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 2013; 10:996-8.
7. McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* 2012; 6:610-8.
8. Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* 2010; 26:266-7.
9. Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Mol Biol Evol* 2009; 26:1641-50.
10. Fujimura KE, Sitarik AR, Havstad S, Lin DL, Levan S, Fadrosh D, et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat Med* 2016; 22:1187-91.

Supplemental Table 1. Selected cohort characteristics for maternal-child pairs included (n=447) and excluded (n=811) from the analyses

Covariate	Included ¹ n=447	Excluded n=811	p-value ²
Child sex			
Male	238 (53.2)	385 (47.5)	0.052
Female	209 (46.8)	426 (52.5)	
Child race			

African American	106 (23.7)	161 (19.9)	0.580
White	269 (60.2)	517 (63.7)	
Hispanic/Latino	28 (6.3)	51 (6.3)	
Other	44 (11.7)	92 (10.2)	
Household income			
< \$40K	121 (30.0)	12 (27.3)	<0.001
\$40K - < \$80K	107 (26.6)	8 (18.2)	
\$80K - ≥\$100K	122 (30.2)	18 (40.9)	
Refused	53 (13.2)	6 (13.6)	
Urban residence	229 (51.2)	474 (58.4)	0.014
Maternal education			
<HS ³ diploma	17 (3.8)	57 (7.0)	<0.001
HS diploma	60 (13.4)	168 (20.7)	
Some college+	370 (82.7)	586 (72.3)	
Mother is married	289 (64.7)	484 (59.7)	0.083
Maternal atopy	261 (59.5)	391 (52.1)	0.014
Prenatal ETS ⁴ exposure	103 (23)	244 (30.1)	0.007
Mom age at birth, mean (sd)	30.0 (5.3)	29.3 (5.2)	0.020
Delivered by cesarean section	163 (36.5)	303 (37.7)	0.657
Breastfeeding at 1 month			
Formula feeding	87 (19.7)	144 (23.7)	0.026
Mixed feeding	283 (64.2)	398 (65.5)	
Exclusive breastfeeding	71 (16.1)	66 (10.9)	
Solid food introduction <4 months	184 (41.2)	225 (37.8)	0.264

¹Children who had food allergy status evaluated by physician panel and ≥ 1 microbiota sample; ²ANOVA for numerical covariates and chi-square test for categorical covariates; ³HS=High School; ⁴ Environmental Tobacco Smoke

Supplemental Table 2. Examining the mediating effect of eczema (AD) by age 2 years in the association between early life microbiota and IgE-FA

Metric	Percent Mediated by AD ¹	p-value
Alpha Diversity		
Richness	1.78%	0.48
Evenness	7.48%	0.46

Diversity (Faith's PD)	4.94%	0.46
Diversity (Shannon's)	6.17%	0.46
Beta Diversity		
Unweighted UniFrac	6.84%	0.44
Weighted UniFrac	5.81%	0.49
Canberra	4.34%	0.46
Bray-Curtis	16.76%	0.46
Maturity		
Bacterial Microbiota-for-Age Z-score (BMAZ)	-2.40%	NA ²

¹*After adjusting for exact age at stool sample collection, child race, maternal history of allergies or asthma, and breastfeeding status at 1-month.*

²*p-value unable to be calculated for negative estimates.*