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

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Abstract

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CONFLICTS OF INTEREST
The authors declare no conflicts of interest.

SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.
**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.

**Keywords**

food allergy; IgE; microbiome

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**1 | INTRODUCTION**

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

Food allergy is a reproducible inflammatory response induced by immunoglobulin E activation of mast cells and basophils upon exposure to a given food. 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. Immune dysregulation is an important contributor to IgE-FA. 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. In IgE-FA, exposure to common food proteins results in an inappropriate T helper 2 cell–mediated response to epitopes of the offending food. 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. In allergy-free individuals, commensal bacteria stimulate responses.
leading to immune tolerance of innocuous allergens.\textsuperscript{5} Aberrant colonization of bacteria in the gut (dysbiosis) increases susceptibility to atopy.\textsuperscript{5} Delayed colonization of gut bacteria leads to irregularities in the development of gut-associated lymphoid tissues, impacting downstream immune pathways.\textsuperscript{6} Associations between gut microbiota composition and food sensitization or IgE-FA are reported in murine and human studies.\textsuperscript{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 cohort\textsuperscript{9} to explore early gut bacterial composition and risk of IgE-FA to egg, milk, or peanut at age 3–5 years.\textsuperscript{9}

\section{METHODS}

\subsection{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.\textsuperscript{9} Methods, eligibility, and recruitment have been described previously.\textsuperscript{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,\textsuperscript{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 \( \geq \) 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 \(-80^\circ\text{C},\) and shipped to UCSF, where they underwent sequencing of the V4 region of the 16S rRNA gene using the Illumina NextSeq (Appendix 1).\textsuperscript{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, gastro-intestinal 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.\textsuperscript{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) \( \geq 1\) specific IgE level \( \geq 0.35\) IU/ml, (2) a positive SPT result, or (3) parental report of infant symptoms potentially related to food allergy plus \( \geq 1\) specific IgE level \( \geq 0.10\) 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.\textsuperscript{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, 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 QIIME and the R vegan package. 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), as well as Canberra and Bray-Curtis dissimilarity (non-phylogenetic). Compositional differences were assessed using PERMANOVA in the R package vegan. 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-values computed to account for multiple testing (FDR-adjusted $p < .05$, significant). OTUs were only tested if they were detected in ≥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. This sparse model was then used to predict age at stool sample collection; BMAZ was calculated as described in Subramanian et al, 2014, 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). 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.

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 $\geq 0.35$ IU/ml (sensitization) as an outcome (Table 2), but a significant effect was observed only for peanut (Table 2).

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.

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.

Children with IgE-FA had significantly lower adjusted bacterial microbiota-for-age z-scores (BMAZ) compared with non-IgE-FA children (Figure 3; $\beta$ (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).

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. Unwanted microbial antigens bound by secretory IgA transferred maternally.
from breastfeeding are handled by the innate immune system. Oligosaccharides in human milk that induce growth of Bifidobacterium and Lactobacillus in the infant gut also induce production of IL-10 and IgA. 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. 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. These infants had higher concentrations of butyric acid than their non-allergic counterparts 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 or results suggestive of delays in gut maturity. In VDAART, investigators reported deficiencies in Clostridium that were significant for both food sensitization and food allergy, but, unlike our analysis, showed subgroup variations for race, mode of delivery, and age at introduction of solid food. 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. The CoFAR study provided additional evidence of decreased fatty acid metabolism, emphasizing potential mechanisms by which risk of IgE-FA is modified.

Our findings contrast with those of studies showing higher abundance of Bifidobacterium associated with reduced risk of atopic disease. 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. 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.

We did not find that atopic eczema, which typically precedes IgE-FA, 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. While no specific bacterial species have emerged in the literature consistently, Bifidobacterium appears in several reports. Zhang, et al. reported decreased abundance of Bifidobacterium associated with eczema only for infants >6 months. Ismail et al. found modulation of eczema risk (among children at high risk of atopy) driven by certain Bifidobacterium species; however, Ta, et al. found atopic eczema was associated with delayed colonization of Bacteroides, but not Bifidobacterium. 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**Abbreviations:**

- **BIC** Bayesian information criteria
- **BSA** Bovine serum albumin
- **CTAB** Cetyltrimethylammonium bromide
- **FDR** False discovery rate
- **HFHS** Henry Ford Health System
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. [PubMed: 30455345]
2. Gupta RS, Springston EE, Warrier MR, et al. The prevalence, severity, and distribution of childhood food allergy in the United States. Pediatrics. 2011;128:e9–e17. [PubMed: 21690110]
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. [PubMed: 29157945]
4. Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. Nature. 2012;489:231–241. [PubMed: 22972296]
5. Yu W, Freeland DMH, Nadeau KC. Food allergy: immune mechanisms, diagnosis and immunotherapy. Nat Rev Immunol. 2016;16:751. [PubMed: 27795547]
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. [PubMed: 24532064]
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. [PubMed: 30643289]
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.
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. [PubMed: 27558272]
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. [PubMed: 32871381]
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. [PubMed: 24854182]
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. [PubMed: 21134568]
13. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. J Allergy Clin Immunol. 2001;107:891–896. [PubMed: 11344358]
14. Caporaso JG, Kuczynski J, Stombaugh J, et al. QiIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335–336. [PubMed: 20383131]
15. Oksanen J, Blanchet G, Friendly M, et al. vegan: Community ecology package. R Package Version. 2016;2(4–4):2017.
16. Lozupone C, Knight R. UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol. 2005;71:8228–8235. [PubMed: 16332807]
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.
18. Liaw A, Wiener M. Classification and regression by randomForest. R News. 2002;2:18–22.
19. Subramanian S, Huq S, Yatsunenko T, et al. Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature. 2014;510:417–421. [PubMed: 24896187]

20. GEEmediate: Mediation Analysis for Generalized Linear Models Using the Difference Method. R package version 1.1.3. Accessed 2019.

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. [PubMed: 32398370]

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. [PubMed: 21720179]

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. [PubMed: 26663491]

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. [PubMed: 25599982]

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. [PubMed: 29321519]

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. [PubMed: 28632934]

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.

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. [PubMed: 30085318]

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.

30. Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. Genome Med. 2016;8:1–11. [PubMed: 26750923]
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.
FIGURE 1.
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.
FIGURE 2.
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
FIGURE 3.
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)

Overall Adjusted β (95% CI) =
-0.70 (-1.08, -0.33)
p-value<0.001
### 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 |
|----------------------------------|---------------|-------------------|---------|
| Child sex                        |               |                   |         |
| Male                             | 23 (52.3)     | 215 (53.3)        | 1.000   |
| Female                           | 21 (47.8)     | 188 (46.7)        |         |
| Child race                       |               |                   |         |
| African American                 | 30 (68.2)     | 239 (59.3)        | .071    |
| White                            | 6 (13.6)      | 100 (24.8)        |         |
| Hispanic/Latino                  | 1 (2.3)       | 27 (6.7)          |         |
| Other                            | 7 (15.9)      | 37 (9.2)          |         |
| Household income                 |               |                   |         |
| <$40K                            | 12 (27.3)     | 121 (30.0)        | .460    |
| $40K – <$80K                     | 8 (18.2)      | 107 (26.6)        |         |
| $80K – =>$100K                   | 18 (40.9)     | 122 (30.2)        |         |
| Refused                          | 6 (13.6)      | 53 (13.2)         |         |
| Urban residence                  | 22 (50.0)     | 207 (51.4)        | .875    |
| Maternal education               |               |                   |         |
| <HS diploma                      | 2 (3.8)       | 15 (7.0)          | .817    |
| HS diploma                       | 4 (13.4)      | 56 (20.7)         |         |
| Some college+                    | 38 (86.3)     | 332 (82.4)        |         |
| Mom age at birth, mean (sd)      | 30.4 (4.8)    | 30.0 (4.8)        | .591    |
| Mother’s marital status          | 29 (65.9)     | 260 (64.5)        | 1.000   |
| Maternal atopy                   | 13 (29.5)     | 165 (41.8)        | .145    |
| Maternal history of allergies or asthma | 16 (36.4) | 109 (27.5)        | .220    |
| Prenatal ETS exposure            | 7 (15.9)      | 96 (23.8)         | .514    |
| Prenatal indoor pet(s)           | 14 (31.8)     | 151 (37.5)        | .51     |
| Delivered by cesarean section    | 15 (34.1)     | 148 (36.7)        | .869    |
| First born child                 | 19 (43.2)     | 155 (38.5)        | .63     |
| Breastfeeding at 1 month         |               |                   |         |
| Formula feeding                  | 7 (15.9)      | 80 (20.2)         | .778    |
| Mixed feeding                    | 29 (65.9)     | 254 (64)          |         |
| Exclusive breastfeeding          | 8 (18.2)      | 63 (15.9)         |         |
| Solid food introduction <4 months | 16 (36.4)     | 168 (41.7)        | .523    |
| Physician diagnosed eczema by age 2 | 16 (37.2) | 72 (19.7)         | .017    |

*p-value* determined by Kruskal-Wallis test for numerical covariates and Fisher’s exact test for categorical covariates.
### TABLE 2

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

| Metric     | Any IgE-FA | Milk IgE-FA | Egg IgE-FA | Peanut IgE-FA |
|------------|------------|-------------|------------|---------------|
|            | Interx\(^3\) | \(\beta\) (95% CI)\(^4\) | \(p\) | Interx\(^3\) | \(\beta\) (95% CI)\(^4\) | \(p\) | Interx\(^3\) | \(\beta\) (95% CI)\(^4\) | \(p\) | Interx\(^3\) | \(\beta\) (95% CI)\(^4\) | \(p\) |
| 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      | −24.4 (−38.37, −10.43) | <.001 |
| 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      | −0.04 (−0.07, −0.01) | .005 |
| 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      | −1.15 (−1.88, −0.42) | .002 |
| 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      | −0.27 (−0.45, −0.1) | .002 |

| Metric     | Any Food Sensitization | Milk Sensitized | Egg Sensitized | Peanut Sensitized |
|------------|------------------------|-----------------|----------------|------------------|
|            | Interx\(^3\) | \(\beta\) (95% CI)\(^4\) | \(p\) | Interx\(^3\) | \(\beta\) (95% CI)\(^4\) | \(p\) | Interx\(^3\) | \(\beta\) (95% CI)\(^4\) | \(p\) |
| Richness   | 0.60       | −2.83 (−12.07, 6.42) | .549 | 0.986      | −1.67 (−11.49, 8.16) | .74  | 0.99       | −7.48 (−18.3, 3.33) | .175 | 0.89       | −17.86 (−29.62, −6.09) | .003 |
| Evenness   | 0.882      | 0.003 (−0.012, 0.018) | .721 | 0.725      | 0.003 (−0.013, 0.019) | .728 | 0.547      | 0.006 (−0.011, 0.024) | .485 | 0.195      | −0.02 (−0.044, 0.004) | .109 |
| Faith’s PD | 0.684      | −0.19 (−0.67, 0.28) | .426 | 0.925      | −0.2 (−0.7, 0.31) | .446 | 0.878      | −0.5 (−1.05, 0.05) | .073 | 0.46       | −0.84 (−1.47, −0.21) | .009 |
| Shannon’s  | 0.967      | 0.002 (−0.091, 0.094) | .973 | 0.687      | 0.006 (−0.091, 0.103) | .903 | 0.576      | 0.009 (−0.101, 0.118) | .874 | 0.267      | −0.14 (−0.285, −0.004) | .045 |

\(^1\) 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 phylogenic diversity.

\(^6\) Shannon’s diversity index.
### TABLE 3
Association between early life microbiome composition and study definition of IgE-mediated food allergy (IgE-FA) and food sensitization

| Outcome                  | Metric                  | 1 Month | 1 Month | 6 Months | 6 Months |
|--------------------------|-------------------------|---------|---------|----------|----------|
|                          |                         | N       | p-value | R²       | N        | p-value | R²       |
| 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    |
|                          | Weighted UniFrac        | 323     | .603    | 0.002    | 282      | .040    | 0.009    |
|                          | Canberra                | 323     | .123    | 0.003    | 282      | .020    | 0.004    |
|                          | Bray-Curtis             | 323     | .370    | 0.003    | 282      | .021    | 0.006    |
| Peanut IgE-FA            | Unweighted UniFrac      | 323     | .182    | 0.004    | 282      | .031    | 0.005    |
|                          | Weighted UniFrac        | 323     | .628    | 0.002    | 282      | .079    | 0.007    |
|                          | Canberra                | 323     | .172    | 0.003    | 282      | .029    | 0.004    |
|                          | Bray-Curtis             | 323     | .182    | 0.004    | 282      | .158    | 0.004    |
| Any food sensitization   | Unweighted UniFrac      | 308     | .259    | 0.003    | 275      | .591    | 0.003    |
|                          | Weighted UniFrac        | 308     | .366    | 0.003    | 275      | .737    | 0.002    |
|                          | Canberra                | 308     | .263    | 0.003    | 275      | .696    | 0.003    |
|                          | Bray-Curtis             | 308     | .039    | 0.006    | 275      | .294    | 0.004    |
| Milk sensitized          | Unweighted UniFrac      | 317     | .412    | 0.003    | 280      | .485    | 0.003    |
|                          | Weighted UniFrac        | 317     | .489    | 0.003    | 280      | .651    | 0.002    |
|                          | Canberra                | 317     | .470    | 0.003    | 280      | .590    | 0.003    |
|                          | Bray-Curtis             | 317     | .148    | 0.004    | 280      | .556    | 0.003    |
| Egg sensitized           | Unweighted UniFrac      | 315     | .175    | 0.004    | 280      | .299    | 0.004    |
|                          | Weighted UniFrac        | 315     | .720    | 0.002    | 280      | .931    | 0.001    |
|                          | Canberra                | 315     | .334    | 0.003    | 280      | .613    | 0.003    |
| Outcome            | Metric          | 1 Month |       | 6 Months |       |
|--------------------|-----------------|---------|-------|----------|-------|
|                    |                 | N       | p-value | N       | p-value |
| Peanut sensitized  | Bray-Curtis     | 315     | .299   | 280      | .339   | 0.004 |
|                    | Unweighted UniFrac | 305     | .041   | 271      | .163   | 0.004 |
|                    | Weighted UniFrac | 305     | .451   | 271      | .813   | 0.002 |
|                    | Canberra         | 305     | .058   | 271      | .375   | 0.004 |
|                    | Bray-Curtis     | 305     | .110   | 271      | .428   | 0.004 |

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.