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Hein M. Tun, D.V.M., Ph.D., Ye Peng, M.Sc., Bolin Chen, Ph.D, Theodore B. Konya, M.P.H., Nadia.P. Morales-Lizcano, Ph.D., Radha Chari, M.D., Catherine J. Field, Ph.D., David S. Guttman, Ph.D, Allan B. Becker, M.D., Piush J. Mandhane, M.D., Ph.D., Theo J. Moraes, M.D., Ph.D., Malcolm R. Sears, M.B., Ch B., Stuart E. Turvey, M.B., B.S., D. Phil, Padmaja Subbarao, M.D., M.Sc., Elinor Simons, M.D., Ph.D., James A. Scott, Ph.D., Anita L. Kozyrskyj, Ph.D.

PII: S0016-5085(21)00523-0
DOI: <https://doi.org/10.1053/j.gastro.2021.03.016>
Reference: YGAST 64207

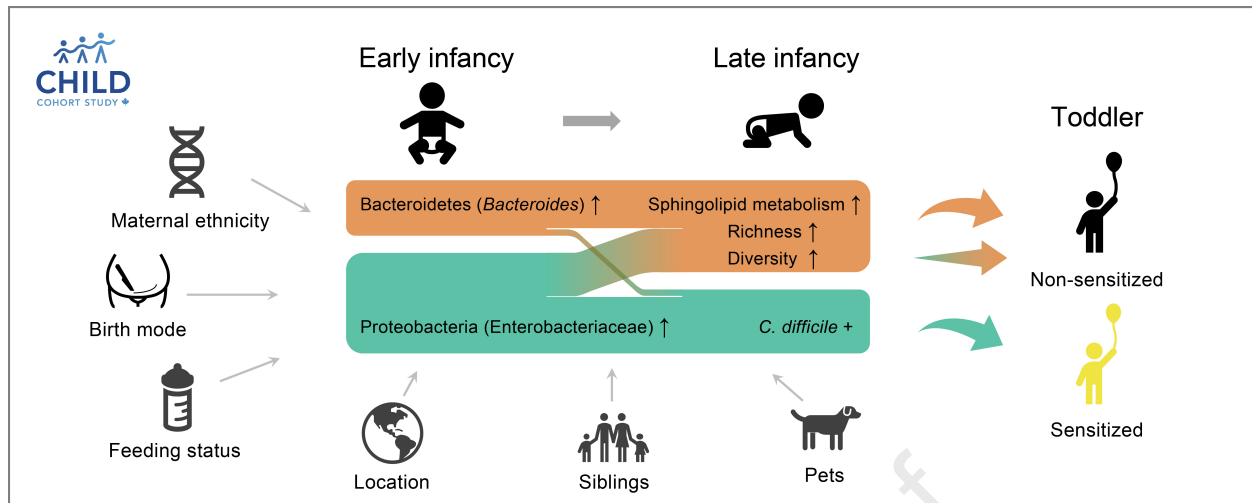
To appear in: *Gastroenterology*
Accepted Date: 8 March 2021

Please cite this article as: Tun HM, Peng Y, Chen B, Konya TB, Morales-Lizcano NP, Chari R, Field CJ, Guttman DS, Becker AB, Mandhane PJ, Moraes TJ, Sears MR, Turvey SE, Subbarao P, Simons E, Scott JA, Kozyrskyj AL, Ethnicity associations with food sensitization are mediated by gut microbiota development in the first year of life, *Gastroenterology* (2021), doi: <https://doi.org/10.1053/j.gastro.2021.03.016>.

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Ethnicity associations with food sensitization are mediated by gut microbiota development in the first year of life

Short title: Infant gut microbiome and ethnicity-associated development of food sensitization

Hein M. Tun, D.V.M., Ph.D.^{1,2*#}, Ye Peng, M.Sc.^{1*}, Bolin Chen, Ph.D.², Theodore B. Konya, M.P.H.³, Nadia. P. Morales-Lizcano, Ph.D.³, Radha Chari, M.D.⁴, Catherine J. Field, Ph.D.⁵, David S. Guttman, Ph.D.⁶, Allan B. Becker, M.D.⁷, Piush J. Mandhane, M.D., Ph.D.², Theo J. Moraes, M.D., Ph.D.⁸, Malcolm R. Sears, M.B., Ch B.⁹, Stuart E. Turvey, M.B., B.S., D. Phil¹⁰, Padmaja Subbarao, M.D., M.Sc.⁸, Elinor Simons, M.D., Ph.D.⁷, James A. Scott, Ph.D.³, Anita L. Kozyrskyj, Ph.D.^{2#}.

¹HKU-Pasteur Research Pole, School of Public Health, University of Hong Kong, Hong Kong SAR, China

²Department of Pediatrics, University of Alberta, Edmonton, AB, Canada

³Dalla Lana School of Public Health, University of Toronto, Toronto, ON, Canada

⁴Department of Obstetrics and Gynecology, University of Alberta, Edmonton, AB, Canada

⁵Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada

⁶Centre for the Analysis of Genome Evolution and Function, University of Toronto, Toronto, ON, Canada

⁷Department of Pediatrics & Child Health, Children's Hospital Research Institute of Manitoba, University of Manitoba, Winnipeg, MB, Canada

⁸Department of Pediatrics, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada

⁹Department of Medicine, McMaster University, Hamilton, ON, Canada

¹⁰Department of Pediatrics, Child & Family Research Institute, BC Children's Hospital,
University of British Columbia, Vancouver, BC, Canada

* Co-first authors

Co-senior authors

Grant support:

This research was specifically funded by grant 227312 from the Canadian Institutes of Health Research Canadian Microbiome Initiative (Dr Kozyskyj). The Canadian Institutes of Health Research and the Allergy, Genes, and Environment (AllerGen) Network of Centres of Excellence provided core support for the Canadian Healthy Infant Longitudinal Development (CHILD) Study. Dr Tun was funded through a Canadian Institutes of Health Research Fellowship and an Alberta Innovates–Postdoctoral Fellowship in Health.

Abbreviations:

CHILD: the Canadian Healthy Infant Longitudinal Development Study

OR: odds ratio

SPT: skin prick testing

IAP: intrapartum antibiotic prophylaxis

SD: standard deviation

C. difficile: *Clostridioides difficile*

sIgA: secretory IgA

ELISA: enzyme-linked immunosorbent assay

PAM: partitioning around medoids

C1: Cluster 1

C2: Cluster 2

PD: phylogenetic diversity

E/B: Enterobacteriaceae/Bacteroidaceae

EBF: exclusive breastfed

P: presence

A: absence

S1P: sphingosine-1-phosphate

VDAART: the Vitamin D Antenatal Asthma Reduction Trial

#Correspondence:

Prof. Anita Kozyrskyj, PhD

Department of Pediatrics, University of Alberta,

3-527 Edmonton Clinic Health Academy, 11405 87 Ave NW, Edmonton, AB, Canada

Phone: 1(780)248-5508, E-mail: kozyrsky@ualberta.ca

Asst. Prof. Hein M. Tun, PhD

HKU-Pasteur Research Pole, School of Public Health, Li Ka Shing Faculty of Medicine, The

University of Hong Kong,

5 Sassoon Road, Pokfulam, Hong Kong SAR

Phone: (852)3917-9944 E-mail: heinmtun@hku.hk

Conflict of interest

The authors declare that they have no conflict of interests.

Author contributions

H.M.T. and A.L.K. developed the study concept and design. R.C., C.J.F., D.S.G., A.B.B., P.J.M., T.J.M., M.R.S., S.E.T., P.S., E.S., and J.A.S. contributed in acquisition of clinical and epidemiological data. T.K. and N.P.M. performed microbiota sequencing and qPCR. B.C. helped in data cleaning and checking. H.M.T. and Y.P processed the data and performed analysis. H.M.T. and Y.P drafted the manuscript. All authors contributed in interpretation, critical revisions, and final approval.

Acknowledgement

The authors would like to thank all the families who took part in this study, and the whole CHILD team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses. Discussions with Dr. Herbert Pang from the University of Hong Kong, and Huahui Ren and Zhenhua Zeng from BGI-Shenzhen have been insightful.

Abstract

Background and aims

Increasing evidence supports the role of early-life gut microbiota in developing atopic diseases, but ecological changes to gut microbiota during infancy in relation to food sensitization remain unclear. We aimed to characterize and associate these changes with the development of food sensitization in children.

Methods

In this observational study, using 16S rRNA amplicon sequencing, we characterized the composition of 2844 fecal microbiota in 1422 Canadian full-term infants. Atopic sensitization outcomes were measured by skin prick tests at age 1 year and 3 years. The association between gut microbiota trajectories, based on longitudinal shifts in community clusters, and atopic sensitization outcomes at age 1 and 3 years was determined. Ethnicity and early life exposures influencing microbiota trajectories were initially examined, and post hoc analyses were conducted.

Results

Four identified developmental trajectories of gut microbiota were shaped by birth mode and varying by ethnicity. The trajectory with persistently-low *Bacteroides* abundance and high Enterobacteriaceae/Bacteroidaceae ratio throughout infancy increased the risk of sensitization to food allergens, particularly peanut at age 3 years by 3-fold (adjusted OR, 2.82; 95% CI, 1.13-7.01); a much higher likelihood for peanut sensitization was found if infants with this trajectory were born to Asian mothers (adjusted OR, 7.87; 95% CI, 2.75-22.55). It was characterized by a deficiency in sphingolipid metabolism and persistent *C. difficile*

colonization. Importantly, this trajectory of depleted *Bacteroides* abundance mediated the association between Asian ethnicity and food sensitization.

Conclusions

This study documented an association between persistently-low gut *Bacteroides* abundance throughout infancy and sensitization to peanut in childhood. It is the first to show a mediation role for infant gut microbiota in ethnicity-associated development of food sensitization.

Keywords: infant gut, microbiota development, atopy, food sensitization

Introduction

Up to 40% of the global population suffers from at least one type of allergy and an estimated 28% of American children between the ages of one to five years are sensitized to at least one food item ¹. These children are at a greater risk of developing not only food allergy but also wheeze, asthma, eczema, and allergic rhinitis when compared to their non-sensitized peers ². Food allergy is often manifested by a default state of immune tolerance that is likely due to antigen exposure, commensal microbiota, and their interactions. Emerging evidence supports the role of gut microbiome in the pathogenesis and course of food allergy ³.

The intestinal microbiome is an enormously complex community which interacts dynamically with the host and early life exposures. Caesarean delivery and formula feeding both disrupt typical mother-to-offspring transmission of microbiota ⁴⁻⁷, whose composition is further modified by the presence of siblings and furry pets in the home ⁸⁻¹⁰. Maternal intrapartum and infant antibiotic treatment have both been implicated in altering pathogenic bacteria, but also commensal microbiota within the gut ^{6, 11}. Divergence of the gut microbiome across populations is not exclusively a function of early life exposures; host variance also plays a role. For instance, a recent study concluded that gut microbial community structure varied significantly among ethnic groups ¹².

Our current knowledge of factors affecting the infant gut microbiome has been enhanced by several recent studies, many of which are founded on cross-sectional data. Even with prospective data collection in a birth cohort study, data may be analysed as a series of cross-sections that uncover unique time-dependent associations. Taking the latter approach, we found the abundance of a key microbial family, Bacteroidaceae, to be reduced in early but not late infancy following caesarean delivery ¹³. Others have observed antibiotic use to significantly diminish phylogenetic richness and diversity immediately after birth, but subsequently accelerate the rate of increase in these measures in later infancy ⁶. A study by

Stockholm *et al.* used a clustering method to describe age-specific composition of gut microbiota during the first year of life; microbiota clusters in 1-year olds were associated with asthma at age 5¹⁴. Though important, these analyses do not provide temporal information on alterations to intestinal microbiota and may fail to distinguish “at-risk” states from healthy chronological variation within the infant’s gut¹⁵.

An accumulating body of evidence supports a relationship between the intestinal microbiome and development of allergic disease, including a handful of publications investigating food sensitivity. More strikingly, gut microbiota from healthy infants has been shown to protect conventionalized mice from cow’s milk allergy¹⁶. Again, most of the existing literature on the early human gut microbiome is based on the analysis of cross-sectional data. In terms of food sensitization, certain microbial markers such as low gut microbiota richness in early infancy, and a higher ratio of Enterobacteriaceae/Bacteroidaceae, in early and late infancy have been found to be predictors^{17, 18}. There has yet to be a longitudinal study of temporal changes in the ecological composition of gut microbiota during infancy as they relate to the development of food sensitization.

With an aim to fill a gap in evidence, using 2844 fecal samples from 1422 infants in the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort, we undertook a study of the developmental trajectories of gut microbiota from early to late infancy (3 months to 12 months of age). The association between gut microbiota trajectories, based on longitudinal shifts in community clusters, and atopic sensitization outcomes at age 1 and 3 years was determined. Host (i.e. ethnicity) and early life exposures influencing microbiota trajectories were initially examined. Once ethnicity was identified as a significant factor, post hoc analyses were conducted to explore the role of microbiota trajectories in ethnicity risk for atopic sensitization.

Methods

Study Design

The Canadian Healthy Infant Longitudinal Development (CHILD) Cohort Study (<http://www.childstudy.ca>) originally recruited 3264 mothers during the third trimester of their pregnancy between 2009 and 2012 (Figure 1A). Enrolment was accepted if their newborns were a singleton live birth at ≥ 35 weeks of gestation with a birth weight of ≥ 2500 g. In vitro fertilized births were excluded to avoid multiple gestation or preterm births. We also excluded home births due to missing documentation of maternal intrapartum antibiotic prophylaxis. Mothers were followed throughout pregnancy and their children assessed from birth to age 3 years (Figure 1B). The present study involved a large subset of 1422 subjects from the cohort. The distributions of most key characteristics between the full cohort and the current study were similar except maternal ethnicity and infant antibiotic exposure in the first year. The exclusion of samples from Toronto site (which includes more Asian mothers) was mainly due to lack of provincial prescription database for assessing antibiotic medication. All potential confounding factors including ethnicity and infant antibiotic exposure were adjusted in downstream analyses.

Outcome measurement and exposure collection

To assess the atopic sensitization at age 1 year, skin prick testing (SPT) was performed by trained staff using 10 standardized inhalant allergens and common food allergens (*Alternaria tenuis*, cat hair, dog epithelium, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, German cockroach, peanut, soybean, egg white, and cow's milk), a positive control (histamine), and a negative control (glycerin). SPT was repeated again at age 3 years with these 10 allergens together with an additional 7 inhalant allergens: penicillium, *Cladosporium*, *Aspergillus fumigatus*, trees, grasses, weeds, and ragweed. If the

child developed a wheal greater than or equal to 2 mm in diameter in response to any allergen, they were considered as positive sensitization for that particular allergen. If a child was positive for glycerin, which served as a negative control, the wheal size for glycerine was subtracted from the wheal sizes from the allergens¹⁹. Positive to atopy was considered if a child sensitized to one of those allergens. However, food or Inhalant sensitization was recognized if a child sensitized to one of the allergens within the particular category. Data on other covariates were obtained from hospital records (mode of delivery, intrapartum antibiotic prophylaxis (IAP)) or from standardized questionnaires completed by mothers (maternal race, maternal asthma and allergy status during pregnancy, presence of siblings, breastfeeding status and infant antibiotic exposure) (Table S1). The Human Research Ethics Boards at the University of Manitoba, University of Alberta, and University of British Columbia approved this study.

Fecal Microbiota Analysis

Fecal samples of infants were collected at both early infancy (mean (SD) age of 3.5 (0.9) months) and late infancy (12.2 (0.3) months) using a standard protocol during a planned home visit and a clinical study visit, respectively. We have indicated methods of sample processing, DNA extraction, amplification and sequencing (the V4 region of 16S ribosomal RNA), and taxonomic assignments in our previous studies^{10, 20} and in online supplementary material.

qPCR for *Clostridioides difficile* (*C. difficile*) Detection

Detailed protocol for detection of *C. difficile* colonization was stated in our recent study²¹ and in online supplementary material.

Quantification of Fecal Metabolites

In a subset of early-infancy (n=505) samples and late-infancy samples (n=179), metabolites were measured using magnetic resonance spectroscopy as described previously²¹. Metabolites were quantified as $\mu\text{mol/gram}$ feces. In this study, 19 metabolites that were detected in >90% of the samples were reported, including acetate, propionate and butyrate.

Quantification of Fecal secretory IgA

In a subset of early-infancy samples (n=507) and late-infancy samples (n=179), fecal secretory IgA (sIgA) was analyzed using the Secretory IgA ELISA (enzyme-linked immunosorbent assay) kit (ELISA, Immundiagnostik AG assay, Bensheim, Germany) and quantified as milligram of sIgA per gram wet weight feces (mg/g) as described previously²¹.

Statistical Analysis

To illustrate the compositional differences in the microbial populations, all samples were clustered using the partitioning around medoids (PAM) clustering algorithm, and the optimal number of clusters was determined by the Calinski-Harabasz index and Silhouette width. Four developmental trajectories of infant gut microbiota were determined according to the shift of cluster membership. Comparisons of continuous data between two groups and more than two groups were made using Wilcoxon's tests and Dunn's tests paired with Benjamini-Hochberg correction, respectively. Comparisons of categorical data were done using Fisher's exact tests. Binary logistic regression models were used to quantify the associations between atopy outcomes and microbiota features (developmental trajectories and colonization of *C. difficile*) or trajectories combined with ethnicity or *C. difficile*, while adjusting for other covariates where appropriate, including age at sampling, location, birth modes, feeding status in early infancy, direct antibiotic exposure during the first 3

months/antibiotic exposure during the first year, solid food introduction, presence and absence of older siblings, and pet exposure (pre- and postnatal), sex, gravida, gestational age, maternal age, maternal race, maternal obesity, maternal diabetes, maternal atopy, maternal food allergy, and maternal asthma. Details of statistical analysis are described in online supplementary methods.

Results

This study included a large sub-sample of 1422 children with fecal microbiota data at two time points (early infancy and late infancy) from the CHILd general population birth cohort (Figure 1A). Among the studied subjects, 90.1% (n=1281) and 99.6% (n=1416) of the samples were successfully analysed for *C. difficile* colonization in early and late infancy respectively, whereas only 89.8% (n=1277) had data for both time points (Table 1). The overall prevalence for atopic sensitization was 12% (n=169) and 12.8% (n=166) at ages 1 and 3 years; 9.5% (n=134) and 5.8% (n=75) were sensitized to food allergens, and 3.3% (n=46) and 10.1% (n=131) to inhalants at age 1 and 3 years respectively.

Gut microbiota converge towards greater diversity and *Bacteroides* abundance in late infancy

In order to understand the developmental programming of gut microbiota, we studied alpha and beta diversity of gut microbiota between early and late infancy. Overall, gut microbiota at the same infant age had lower divergence than between different ages ($p < 0.001$), exhibiting lower beta diversity between samples or more similar community composition (Figure 2A). Compared to early infancy, gut microbiota of late infancy had lower beta-diversity and intra-individual variability ($p < 0.001$), reflecting the convergence trait of gut microbiota over the first year of life (Figure 2A). In general, microbiota in late infancy had higher Chao1 species richness and Shannon diversity than early infancy ($p < 0.01$) (Figure 2A). Regarding taxonomy, late infancy microbiota manifested higher diversity across all phyla tested, greater Bacteroidetes and Firmicutes species richness, but lower Proteobacterial richness (Table S10). Compared to early infancy, late infancy microbiota had an enhanced abundance of Bacteroidetes and Firmicutes, and reduced abundance of Proteobacteria and Actinobacteria (Figure S1). At the genus level, late infancy microbiota

were enriched with *Bacteroides*, *Faecalibacterium*, *Lachnospira*, *Prevotella*, unclassified Lachnospiraceae and unclassified Clostridiales, but depleted with *Clostridium*, *Veillonella*, *Bifidobacterium* and unclassified Enterobacteriaceae (Figure 2B).

The C1-C1 microbiota trajectory: persistently-low *Bacteroides* in early and late infancy

The PAM clustering method divided all fecal samples (n=2844) into two distinct microbiota clusters (Figure 2C) (Figure S2). 75.5% of samples in PAM-Cluster 1 (C1, n=993) were collected in early infancy (Figure 2C). The PAM-Cluster 2 (C2, n=1851) was dominated by late infancy samples (63.7%, n=1179). In early or late infancy, most infant born vaginally without intrapartum antibiotic prophylaxis (IAP) possessed the gut microbiota of C2 (Figure 2D and E), which was dominated by genus *Bacteroides* (Figures 2F-H).

Four trajectories of gut microbiota clusters across early and late infancy were identified: C1-C1, C1-C2, C2-C1 and C2-C2. Among these, C2-C2 was the most common trajectory (n = 650, 45.7%), followed by C1-C2 (n = 529, 37.2%), C1-C1 (n = 221, 15.5%) and C2-C1 (n = 22, 1.5%) (Figure 3A). Infants belonging to the C2-C2 trajectory were enriched with *Bacteroides* from early to late infancy. The C1-C2 trajectory represented higher abundance of *Bacteroides* in late but not early infancy. Persistently-low levels of *Bacteroides* but higher abundance of *Veillonella*, *Bifidobacterium* and Unclassified genus of Enterobacteriaceae were characteristic of the C1-C1 trajectory, while genus *Bacteroides* was depleted only in late infancy among C2-C1 subjects (Figures 2F-H and Figure 3B).

Compared to C2-C2, the C1-C1 trajectory was characterized by persistently lower Chao1, observed OTUs and PD whole tree (Faith's phylogenetic diversity) indices for the whole microbial community, as well as within the Bacteroidetes phylum (Table S10). Shannon and Simpson diversity of Bacteroidetes in this trajectory was also lower in late infancy (Table S10). Compared to other trajectories, the C1-C1 trajectory had the highest

Enterobacteriaceae/Bacteroidaceae (E/B) ratio in both early and late infancy (Figure S3A).

The trend for the E/B ratio was consistent at the genus level for the unclassified

Enterobacteriaceae/*Bacteroides* ratio (Figure S3B).

The C1-C1 microbiota trajectory is more common in Asian infants

We next investigated ethnic and pre- and postnatal factors linked to the developmental trajectories of gut microbiota. Compared to C2-C2, the C1-C1 trajectory was composed of a greater proportion of infants born to Asian mothers (23.1%, mainly Chinese and Filipino) than to Caucasian mothers ($p < 0.05$, Table 1). Unlike other trajectories, the C1-C1 trajectory consisted of relatively fewer infants born vaginally without IAP, more infants born by emergency cesarean section and a greater percentage of infants with no older siblings. A higher proportion of exclusive breastfed (EBF) infants belonged to the C1-C1 and C1-C2, and they were less likely to have pet exposure, than the C2-C2 trajectory (Table 1).

The C1-C1 microbiota trajectory constitutes risk for atopic sensitization

Logistic regression modelling was employed to test associations between microbiota cluster or trajectory membership with atopic sensitization, including sensitization to food and inhalant allergens at ages 1 year and 3 years (Figure 4A and Table S11-16). Compared with C2-C2 and C1-C2, being in the C1-C1 trajectory was associated with higher odds of atopic sensitization at age 3 years (versus C2-C2, Model 2: adjusted OR, 1.90; 95% CI, 1.15-3.14; versus C1-C2, Model 2: adjusted OR, 2.38; 95% CI, 1.43-3.96, Figure 4A, Table S11-16). These associations were diminished by pre-postnatal factors and maternal ethnicity (model 3 Figure 4A, Table S11-12).

Compared to C2-C2 and C1-C2, infants in the C1-C1 trajectory were twice more likely to have food sensitization at age 3 years (versus C2-C2, Model 2: adjusted OR, 2.34;

95% CI, 1.20-4.56; versus C1-C2, Model 2: adjusted OR, 2.60; 95% CI, 1.33-5.09) (Figure 4A, Table S13-14). Being in the C1-C1 trajectory also showed increased risk for sensitization to inhalants at age 3 years but only versus the C1-C2 trajectory (Table S15-16). Among food allergens, only peanut sensitization at age 3 years was associated with membership in the C1-C1 trajectory (versus C2-C2, Model 3: adjusted OR, 2.82; 95% CI, 1.13-7.01; versus C1-C2, Model 3: adjusted OR, 2.01; 95% CI, 0.85-4.78) (Figure 4A, Table S17). The cross-sectional (Table S18-31), cluster C1 in late infancy was associated with food and peanut sensitization, but odds ratios were mostly non-significant than for the C1-C1 trajectory, likely due to dilution by the non-significant C2-C1 trajectory (Figure 4A, Table S13 vs. Table S23, Table S14 vs. Table S25, Table S17 vs. Table S31). No associations were found for the C1 cluster in early infancy.

In a sensitivity analysis, compared to infants who became sensitized to peanut in age three years, non-sensitized infants had persistently higher *Bacteroides* (Model 2, p value = 0.044), lower unclassified Enterobacteriaceae (Model 2, p value = 0.001) and lower E/B ratio throughout infancy (Model 2, p value = 0.013) (Table S32).

The C1-C1 trajectory mediates Asian ethnicity risk for food and peanut sensitization

Since maternal ethnicity was significantly associated with trajectory membership and sensitization outcomes, and diminished risk estimates for the C1-C1 trajectory, we performed 4 sets of post hoc analyses: i) ethnicity-specific modelling, ii) tests of ethnicity-trajectory interactions, iii) microbiota effect modification of ethnicity-outcome associations, and iv) microbiota mediation of ethnicity-outcome associations. The C1-C1 trajectory was associated with the food sensitization status of Caucasian 3-year olds (versus C2-C2, Model 3: adjusted OR, 2.67; 95% CI, 1.08-6.57; versus C1-C2, Model 3: adjusted OR, 3.42; 95% CI, 1.30-8.98, Figure S5A). Membership in the C1-C1 trajectory increased risk for peanut sensitization at

age 3 years for both Caucasian and Asian infants, but only when compared to C2-C2 (Caucasian, Model 3: adjusted OR, 5.41; 95% CI, 1.51-19.46; and Asian, Model 3: adjusted OR, 8.35; 95% CI, 1.15-60.84, Figure S5B). To enable comparison across ethnic groups, we tested a 4-category variable based on microbiota trajectory (C1-C1, non-C1-C1) and maternal ethnicity (Asian, non-Asian). The magnitude of the risk for food sensitization in infants with the C1-C1 trajectory, born to Asian mothers (Model 3: adjusted OR, 4.67; 95% CI, 1.94-11.21), was twice that for infants born to non-Asian mothers (Model 3: adjusted OR, 2.04; 95% CI, 0.99-4.19), with non-C1-C1 and non-Asian mothers as the reference category (Figure 4B, Table S34). For peanut sensitization, an even higher magnitude of association was observed for the Asian C1-C1 trajectory: Model 3 adjusted OR 7.87, 95% CI, 2.75-22.55) (Figure 4B, Table S35).

Maternal ethnicity was not found to be an effect modifier, either in the multiplicative scale or additive scale, for excess risk of food or peanut sensitization at age 3 years with the C1-C1 trajectory (Table S36-37). On the other hand, the association between ethnicity and infant food and peanut sensitization outcomes at age 3 years was significantly mediated by the C1-C1 microbiota trajectory (Figure 4C, Table S38-39).

C. *difficile* colonization adds to C1-C1 trajectory risk for peanut sensitization

Infants in the C1-C1 trajectory were more likely to be colonized with *C. difficile* in late infancy (Figure 4D). Although trajectories of *C. difficile* colonization alone were not associated with food sensitization (Figure 4E, Table S40), *C. difficile* colonization in early and late infancy (P-P) added significant extra risk for food and peanut sensitization in C1-C1 infants at age 3 years (For food sensitization, Model 3: adjusted OR, 5.69; 95% CI, 1.62-19.99 and for peanut sensitization, Model 3: adjusted OR, 5.89; 95% CI, 1.16-29.87) (Figure 4E, Table S41-42).

The C1-C1 microbiota trajectory: deficiency in sphingolipid metabolism

Among predicted microbiota functions (Figure S6), sphingolipid metabolism and three glycosphingolipid biosynthesis-related functions (ganglio series, globo series and, lacto and neolacto series) were enriched in late infancy with the C2-C2 versus other trajectories (Figure S6B). Relative abundances of these four predicted functions were significantly lower in Asian infants than in Caucasian infants (Figure S7-S10). Such variation was statistically significant in infants of the C1-C1 but not the C2-C2 trajectory (Figure S7-S10). In early infancy, significantly higher fecal succinate was seen in C2-C2 infants compared to C1-C1 and C1-C2 infants (Table S43). In late infancy, infants of the C1-C1 trajectory had significantly lower levels of fecal propionate, valerate, and isobutyrate, but higher acetate levels than C2-C2 infants (Table S43). Fecal sIgA levels did not differ between C1-C1 and other trajectories in both early and late infancy (Figure S11).

Discussion

In 1422 infants with longitudinal profiling from our general population birth cohort, we found the trajectory of gut microbiota assemblage during the first year of life to be key in identifying risk for atopic sensitization. Infants with a gut microbiota cluster depleted in *Bacteroides* in early and later infancy, labelled the C1-C1 trajectory, had a 1.6 fold greater odds of developing atopic sensitization relative to the *Bacteroides*-abundant C2-C2 trajectory. The C1-C1 microbiota cluster was also enriched with *Veillonella* and Enterobacteriaceae species, and it was more common after caesarean delivery. The association between the C1-C1 trajectory and atopic sensitization was observed for food and inhalant sensitization, but it was strongest in magnitude for peanut sensitization at age 3 years (adjusted OR, 2.82; 95% CI: 1.13-7.01). Our findings build on smaller-scale studies of our own and others on the E/B ratio predicting risk for food sensitization^{17, 18}, and are consistent with asthma development in a Danish birth cohort¹⁴. Moreover, we found the C1-C1 trajectory to include twice as many Asian than non-Asian Canadian infants. In its presence, Asian infants were substantially more likely to develop peanut sensitization, independent of environmental and lifestyle factors. Children of Asian ancestry are most likely to be at risk of food-induced anaphylaxis in other countries compared to non-Asian children and children born in Asia who migrated later^{22, 23}. Finally, we confirmed the C1-C1 trajectory to be a mediator for peanut and overall food sensitization for infants of Asian ethnicity.

A key characteristic of microbial function in the C1-C1 trajectory with depleted *Bacteroides* abundance points to microbiota pathways for food sensitization. Notably in later infancy, the metabolism of sphingolipids and pyridoxine (vitamin B6) cofactors for rate-limiting enzymes in sphingolipid metabolism were less abundant in gut microbiota of the C1-C1 versus C2-C2 trajectory. Sphingolipids, cellular membrane proteins of the skin, gut and brain, control movement of cytokines and other signalling molecules of the immune system²⁴.

Both de novo sphingolipid synthesis and metabolism have been linked to atopic disease²⁵. Only Bacteroidetes and a limited set of other gut microbiota have the capacity to synthesize and metabolize sphingolipids²⁶. In the VDAART cohort, sphingolipid metabolism was reported to mediate the association between *Bacteroides* abundance and food sensitization²⁷. We observed this as well and contribute additional evidence for interactions with host genetics. Infants of Asian ethnicity exhibiting the C1-C1 trajectory had significantly lowered sphingolipid metabolism compared to Caucasian infants, as well as a reduction in biosynthesis paths that recycle sphingolipids. Intestinal lipase is also an enzyme that breaks down sphingolipids to intermediates that can be recycled by *Bacteroides*²⁴. Hence, we propose that lactase deficiency, common in Asians²⁸, worsens the sphingolipid deficiency observed when sphingolipid-producing *Bacteroides* are depleted.

The Bacteroidetes possess pyroxidine-dependent enzymes to produce and metabolize sphingolipids, and for this purpose, can utilize exogenous odd-chain fatty acids available from breast milk fatty acids or the breakdown of milk lipids²⁹⁻³¹. Further, *Bacteroides* are able to synthesize odd-chain fatty acids from the elongation of propionate, a short-chain fatty acid they commonly produce^{32, 33}. Valerate and ethanol are consumed in the elongation process of propionate. Hence, the resultant pattern of fecal metabolites found in 12-month old infants (higher ethanol but reduced propionate and valerate levels) was indicative of reduced synthesis of odd-chain fatty acids with depleted abundance of *Bacteroides*. Few differences in metabolites were seen among trajectories at age 3 month; we attribute this to the influence of breastfeeding. Sphingomyelin is present in breast milk, detected in the stool of vaginally-born breastfed infants and slowly degraded to sphingosine in the colon^{24, 34}. Sphingosine is a building block for other gangliosides²⁶ and it also facilitates the absorption of milk protein³⁵. As shown experimentally, sphingomyelin administration increases colon availability of milk protein fragments, which can be metabolized to propionate by microbiota more abundant in

the C1-C1 trajectory - *Ruminococci*, *Veillonella*, Enterobacteriaceae or *C. difficile*^{21, 33, 36}.

This may explain why propionate was not reduced to the same extent at 3 months as it was at 1 year, resulting in a lesser impact on odd-chain fatty acid production, and lack of observed difference in sphingolipid metabolism between trajectories at age 3 months. It is also conceivable that lipase deficiency and greater colonic dumping of milk protein fragments in Asian infants may have further promoted protein degradation by microbiota enhanced in the C1-C1 trajectory, and lactate accumulation that is detrimental to the growth of *Bacteroides*³⁷. In contrast, when early exclusive breastfeeding following cesarean delivery leads to the recovery of *Bacteroides* abundance in late infancy¹³, akin to the C1-C2 trajectory with its lesser representation of Asian infants, the risk for atopy is lower than the C1-C1 trajectory.

Asymptomatic colonization by *C. difficile* is common in infants; its presence in the gut can stimulate eosinophil influx³⁸ and is found to be associated with food allergy in some populations³⁹. The colonization of *C. difficile* in late infancy was greatest among infants in the C1-C1 trajectory, in conjunction with the enrichment of several Firmicutes microbiota, a gut dysbiosis observed when *C. difficile* is induced experimentally²¹. Depletion of genus *Bacteroides* in the C1-C1 trajectory would also prevent competitive inhibition of *C. difficile* growth⁴⁰. Although *C. difficile* colonization alone did not predict peanut sensitization, trajectories of either persistent colonization or only late infancy colonization further enhanced atopy risk. *C. difficile*'s production of the pro-inflammatory metabolite cresol, induction of microbial dysbiosis or disruption of the intestinal barrier may be key factors in the onset of food sensitization⁴¹⁻⁴³.

Our observed trajectories of microbiota enterotypes, namely the dominance of Proteobacteria relative to the Bacteroidetes in the early microbiota C1 cluster, were similar to those reported in cohorts across the globe^{15, 44-46}. Depletion of Bacteroidetes in the cesarean section born infants has been widely reported across different populations, including the

CHILD cohort^{45, 47}. Consistently, most vaginally-born infants in our study were clustered into C2, with higher abundance of Bacteroidetes in early infancy. Evidence is emerging that reduced transmission of maternal Bacteroidetes during birth increases risk for infant colonization with pathobionts⁴⁸. In our study infants, reduced *Bacteroides* abundance was also associated with enhanced *C. difficile* colonization.

Infants exhibiting cluster C1 microbiota at 3 months of age were not at risk for food sensitization if their gut microbial composition converted to cluster C2 later in life. This result underpins the importance of studying longitudinal microbial development throughout the first year of life, the window of opportunity for microbiota and immune development^{11, 49}. Our study had additional strengths, including the use of high-throughput deep sequencing and metabolomics to define developmental trajectories of gut microbiota in a large, general population birth cohort, based on fecal samples collected at two critical time points in early and late infancy. The large sample size for longitudinal microbiota data enabled statistical modelling and sensitivity analyses to verify that observed associations were not attributable to confounding bias. The sensitivity analyses integrated both microbiota and genetic factors to test associations with food sensitization, from which came new evidence of microbiota pathways for ethnic predisposition to food sensitization. On the other hand, use of 16S rRNA sequencing in this study may limit our understanding of the functional capacities of microbiota, their low abundant members, and the roles of other non-bacterial microbiota (e.g. mycobiome and virome).

In conclusion, this study revealed different developmental trajectories of the gut microbiota in the first year of life based on sequential fecal microbiota samples of 1,422 subjects from the CHILD birth cohort. We identified influential genetic, environmental, other early-life factors shaping the trajectories, in which birth mode played the most significant role. In this large prospective cohort, we reported risk of food sensitization in infants with

persistently low levels of *Bacteroides*, with a greater risk for infants born to Asian mothers or colonized by *C. difficile*. These findings further support the future development of microbiota-based preventive or therapeutic intervention strategies for food allergy in toddlers.

Data Sharing Statement

The datasets used and/or analysed in the current study are available from the corresponding author on reasonable request.

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Author names in bold designate shared co-first authorship.

Figures

Figure 1. Study design. (A) Subjects inclusion and exclusion from the CHILd cohort. This study involved a large subset of 1,422 subjects from the CHILd cohort, which originally recruited 3455 mothers. (B) A schematic diagram for data collection. Questionnaires were collected prenatally, as well as in early (~3 months of age) and late (~12 months of age) infancy. Atopic sensitizations were measured by skin prick tests at age 1 year and age 3 years. Stool samples were collected in early and late infancy, and analysed by amplicon sequencing of the V4 region of 16S rRNA, *Clostridioides difficile* (*C. difficile*)-targeted qPCR, magnetic resonance spectroscopy for quantification of metabolites and ELISA for quantification of secretory IgA.

Figure 2. Development of gut microbiota and its influential factors of the study cohort in the first year of life. (A) Distributions of beta diversity between different groups. *P* values were given by two-sided Wilcoxon's tests. (B) Differential genera between early and late-infancy microbiota. (C) PCoA of all gut microbial communities based on genus-level JSD matrix. Sampling times (early and late infancy) were denoted by different shapes. Cluster memberships assigned by PAM clustering were denoted by different colors. Samples from the time point and belonging to the same cluster were enclosed in eclipses. (D) CCA plot showing factors influencing early infancy gut microbiota clusters. (E) CCA plot showing factors influencing late infancy gut microbiota clusters. (F) Differential genera between clusters for all samples. (G) Differential genera between clusters in early infancy. (H) Differential genera between clusters in late infancy.

Figure 3. Developmental trajectories of gut microbiota in the first year of life and associated dominant genera. (A) Four developmental trajectories of gut microbiota in the study cohort. (B) A heatmap showing dominant genera (top 10) in each of the trajectories.

Figure 4. Associations of atopic sensitization with microbiota developmental trajectories, genetic background of infants and *C. difficile* colonization. (A) Associations of major atopic outcomes with cluster membership at early and late infancy and developmental trajectories of gut microbiota. (B) Association between food/peanut sensitizations at age 3 years and infant's microbiota developmental trajectories plus genetic background. (C) Associations between ethnicity and sensitization outcomes mediated by microbiota developmental trajectories (reference group: non-Asian, non-C1-C1 as the reference group). 95% CI generated using 1000 bootstraps. (D) Prevalence of *C. difficile* colonization in early and late infancy stratified by trajectories. Fisher's exact tests with C1-C1 as the reference group. (E) Association of peanut sensitization in age 3 years with *C. difficile* colonization and microbiota developmental trajectories (reference group for the top two panels: no *C. difficile* colonization (A-A); reference group for the bottom two panels: non-C1-C1, A-A). Odds ratios were presented with 95% confidence intervals. Those that were statistically significant were shown in orange. The vertical dashed lines were located at OR = 1 for reference.

Table 1. Population characteristics stratified by developmental trajectory of gut microbiota.

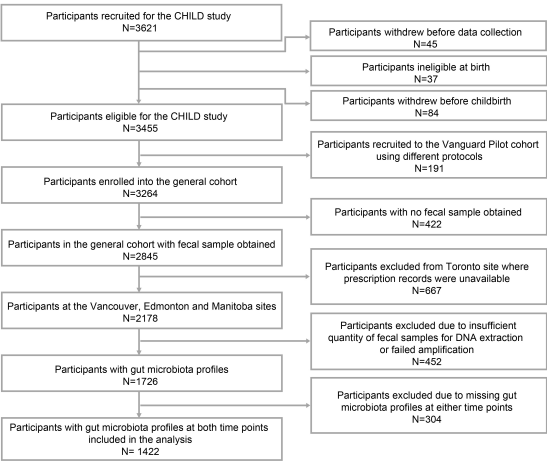
	C2-C2 (n = 650)	C1-C2 (n = 529)	C1-C1 (n = 221)	C2-C1 (n = 22)	<i>p</i> value
Gestation age (weeks)	40 (39, 40)	39 (38, 40)	39 (38, 40)	39 (38, 40)	0.084
Gravida	2 (1, 3)	2 (1, 3)	2 (1, 2)	2 (1.2, 3)	<0.001
Location					0.004
Vancouver	212 (32.6)	172 (32.5)	79 (35.7)	8 (36.4)	
Edmonton	118 (18.2)	122 (23.1)	59 (26.7)	9 (40.9)	
Winnipeg	320 (49.2)	235 (44.4)	83 (37.6)	5 (22.7)	
Maternal pre-pregnancy weight status					0.682
Normal weight	371 (59.5)	317 (62.6)	133 (62.7)	14 (63.6)	
Overweight or Obese	253 (40.5)	189 (37.4)	79 (37.3)	8 (36.4)	
Maternal prenatal diabetes					0.627
No	613 (95.5)	501 (94.9)	210 (95.5)	20 (90.9)	
Yes	29 (4.5)	27 (5.1)	10 (4.5)	2 (9.1)	
Maternal prenatal atopy					0.912
No	101 (16.1)	82 (15.7)	33 (15.2)	2 (9.1)	
Yes	528 (83.9)	439 (84.3)	184 (84.8)	20 (90.9)	
Maternal prenatal food allergy					0.659
No	477 (74.3)	393 (74.4)	160 (72.7)	14 (63.6)	
Yes	165 (25.7)	135 (25.6)	60 (27.3)	8 (36.4)	
Maternal prenatal asthma					0.078
No	602 (92.9)	484 (91.7)	201 (91)	17 (77.3)	
Yes	46 (7.1)	44 (8.3)	20 (9)	5 (22.7)	
Maternal ethnicity					<0.001
Caucasian	507 (79.3)	423 (80.4)	147 (66.5)	14 (63.6)	
Asian	64 (10)	52 (9.9)	51 (23.1)	4 (18.2)	
Others	68 (10.6)	51 (9.7)	23 (10.4)	4 (18.2)	
Sex					0.964
Female	301 (46.3)	249 (47.1)	106 (48)	11 (50)	
Male	349 (53.7)	280 (52.9)	115 (52)	11 (50)	
Birth mode					<0.001
Vaginal, IAP-	440 (69.8)	210 (40.5)	80 (37)	11 (50)	
Vaginal, IAP+	140 (22.2)	113 (21.8)	52 (24.1)	8 (36.4)	
Cesarean, scheduled	18 (2.9)	94 (18.1)	19 (8.8)	3 (13.6)	

Cesarean, emergency	32 (5.1)	101 (19.5)	65 (30.1)	0 (0)	
Breastfeeding status (0-3 months)					0.015
Formula fed	117 (18)	81 (15.3)	26 (11.8)	2 (9.1)	
Partially breastfed	186 (28.6)	130 (24.6)	46 (20.8)	7 (31.8)	
Exclusively breastfed	347 (53.4)	318 (60.1)	149 (67.4)	13 (59.1)	
Direct antibiotic exposure (0-12 months)					0.851
No	455 (80)	351 (78.5)	159 (81.5)	15 (83.3)	
Yes	114 (20)	96 (21.5)	36 (18.5)	3 (16.7)	
Siblings					<0.001
No	274 (43.1)	245 (47.6)	146 (68.2)	11 (50)	
Yes	362 (56.9)	270 (52.4)	68 (31.8)	11 (50)	
Solid food introduction (0-3 months)					0.242
No	588 (93)	484 (92.5)	209 (95.4)	19 (86.4)	
Yes	44 (7)	39 (7.5)	10 (4.6)	3 (13.6)	
Household pet exposure					0.669
No	302 (46.5)	276 (52.2)	118 (53.4)	11 (50)	
Only Prenatal	45 (6.9)	34 (6.4)	14 (6.3)	2 (9.1)	
Both Pre- and Postnatal	293 (45.1)	213 (40.3)	88 (39.8)	9 (40.9)	
Only Postnatal	9 (1.4)	6 (1.1)	1 (0.5)	0 (0)	
<i>C. difficile</i> colonization (absent (A) and present (P) colonization at early and late infancy)					0.028
A-P	182 (29.2)	113 (25.2)	60 (32.1)	2 (9.1)	
P-P	87 (13.9)	59 (13.2)	36 (19.3)	3 (13.6)	
P-A	89 (14.3)	86 (19.2)	24 (12.8)	3 (13.6)	
A-A	266 (42.6)	190 (42.4)	67 (35.8)	14 (63.6)	

Continuous data were shown in median (1st quartile, 3rd quartile), and differences were evaluated using Kruskal-Wallis tests. Categorical data were shown in n (% proportion), and differences were evaluated using Fisher's exact tests.

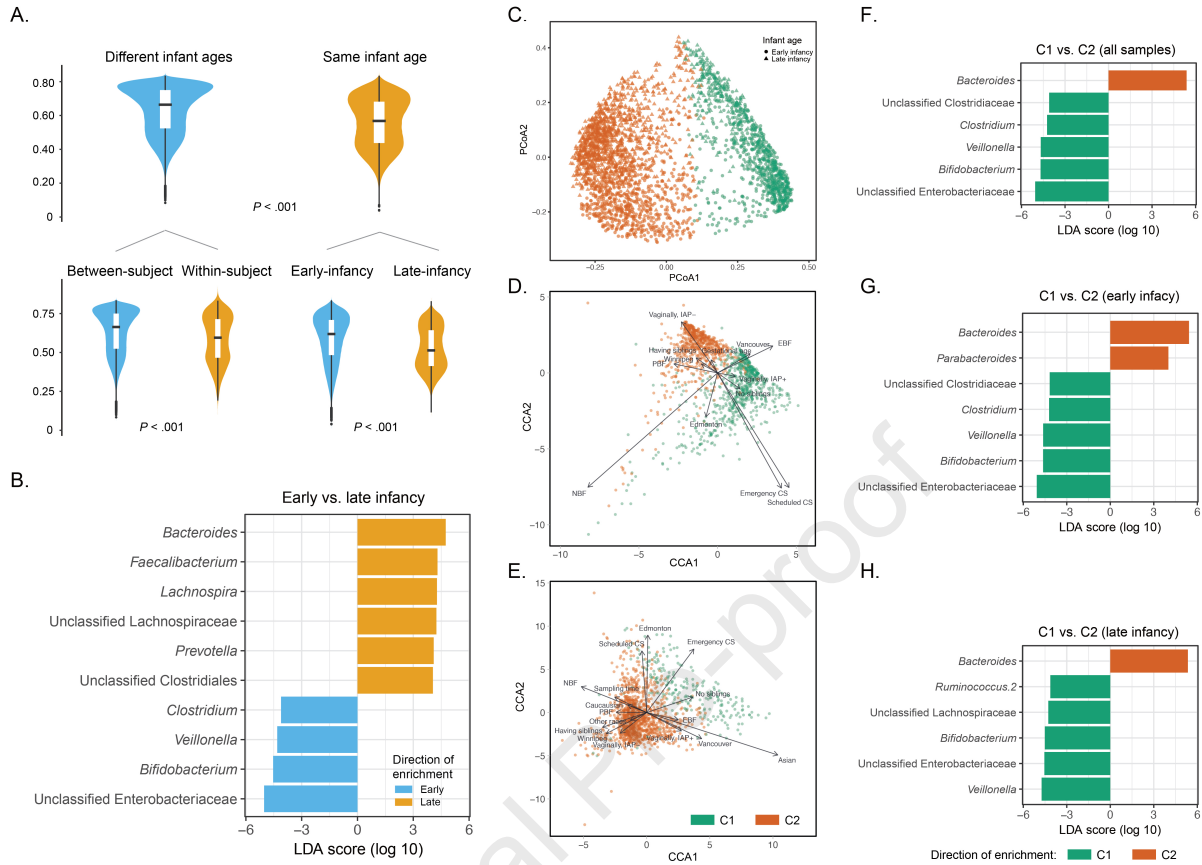
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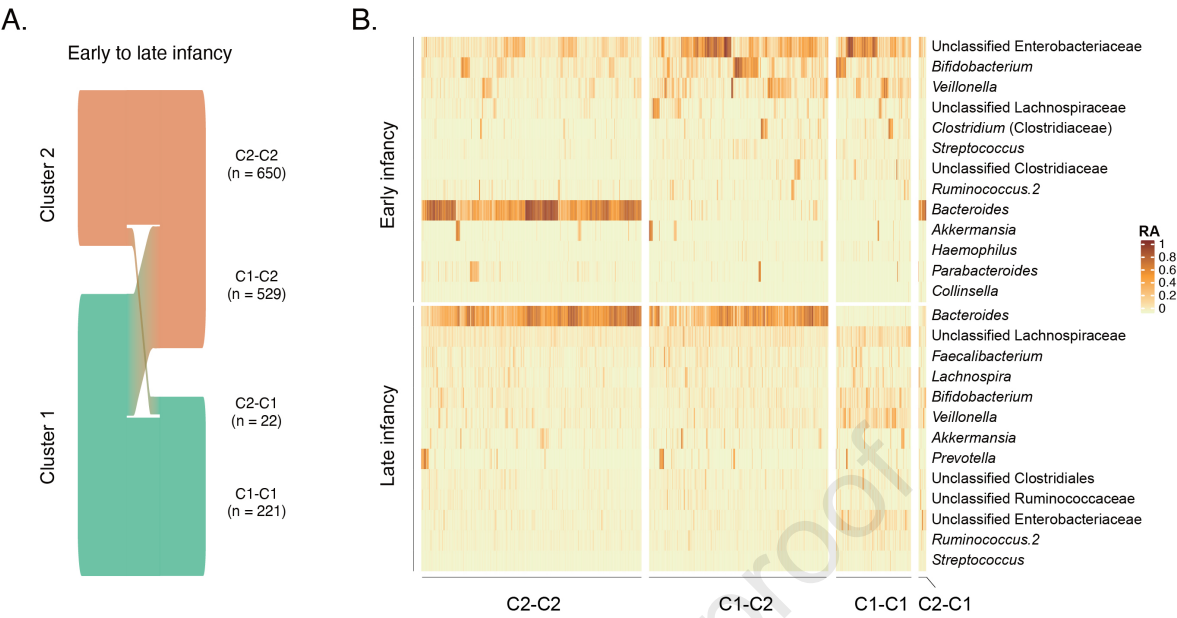
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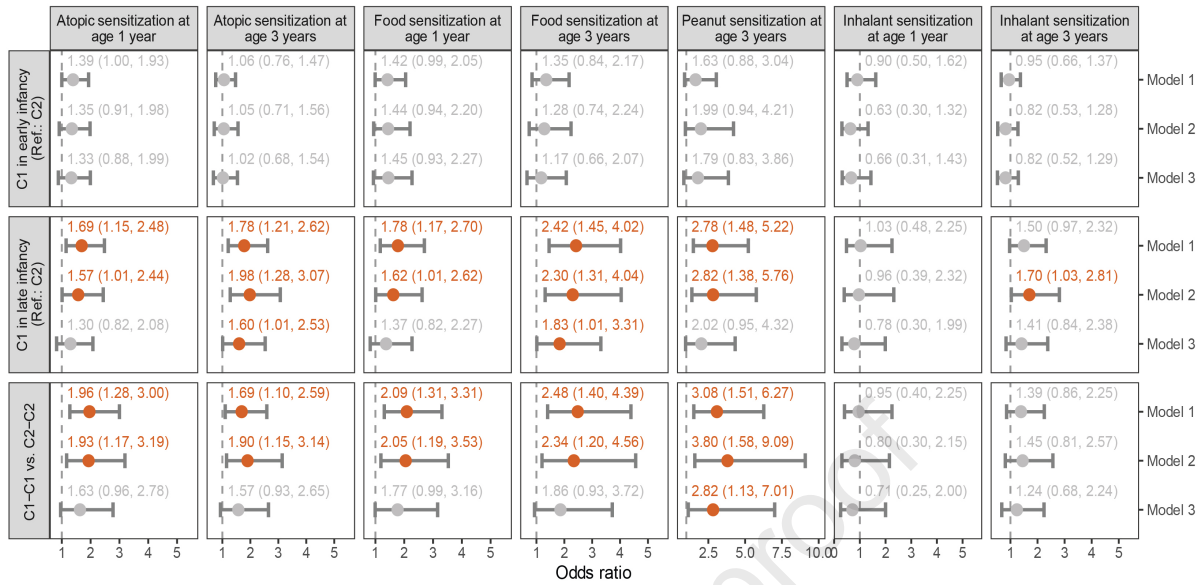
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Time	Prenatal	3-month	12-month	3-year
Questionnaires				
Diagnosis for atopic sensitizations (skin prick tests)				
Gut microbiota analysis (amplicon sequencing, V4 region of 16S rDNA)				
<i>C. difficile</i> colonization (targeted qPCR)				
Quantification of metabolites (magnetic resonance spectroscopy)				
Quantification of secretory IgA (ELISA)				

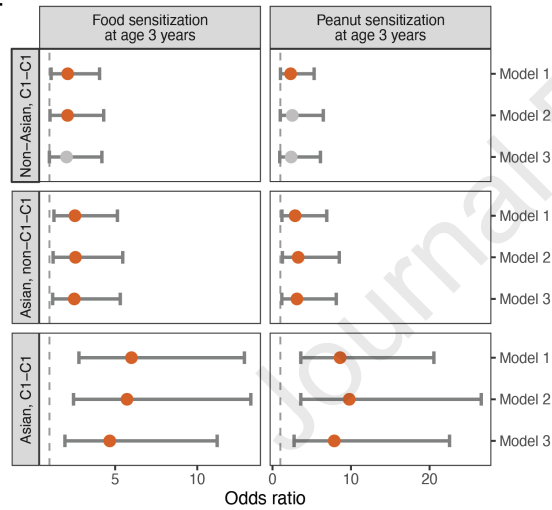




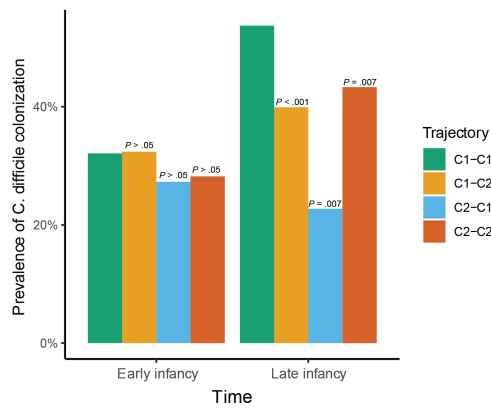
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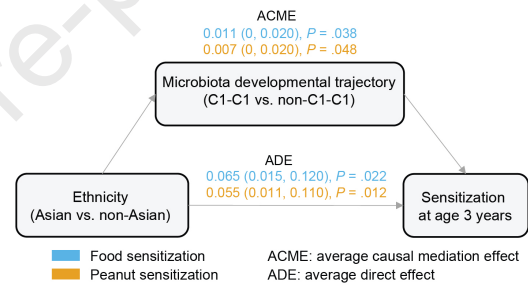
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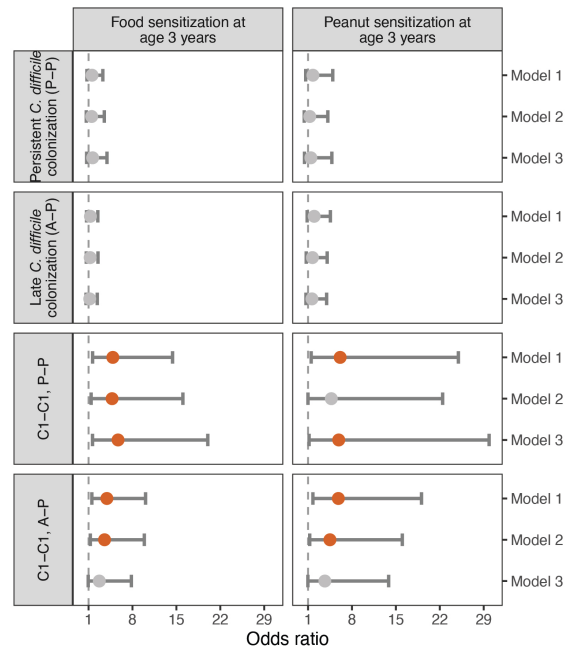
D.



C.



E.



Supplementary Material

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Supplementary Methods

Fecal Microbiota Analysis

Briefly, collected fecal samples were refrigerated after collection and during transport and kept at -80°C until analysis. DNA was isolated from 80 to 200 mg of fecal material using the QIAamp DNA Stool Mini kit (Qiagen, Venlo, the Netherlands) as per the manufacturer's instructions. DNA was then amplified to target V4 hypervariable region of the bacterial 16S rRNA gene using universal primers: V4-515f and V4-806r. The reverse primers were barcoded uniquely (denoted as consecutive Xs) for each sample for multiplexing. Each 25 μl PCR mixture contained 12.5 μl 2x Kapa2G Hotstart mix (Kapa Biosystems, Wilmington, MA), 0.6 μM of both forward and reverse primers and 2 μl genomic DNA (5 ng/ μl). PCR reactions for each sample were performed in triplicate with a negative control in each run with the following conditions: an initial denaturation step for 3 min at 94°C , 20 cycles of denaturation for 30 s at 94°C , annealing for 30 s at 50°C and an extension step for 30 s at 72°C . 100 ng of pooled PCR product from each sample was concentrated using an Amicon Ultra-4 30K centrifugal filter (Millipore, Billerica, MA, USA), run on a 1.4% agarose gel, extracted and cleaned with the GENE-CLEAN Turbo Kit (MP Biomedicals Inc, Solon, OH, USA). Paired-end sequencing of the pooled PCR amplicons was performed on the Illumina Miseq platform.

Bioinformatic Analysis

As per the QIIME pipeline (v1.9.0)¹, forward and reverse sequencing reads were assembled into a final length of 144 bp (singletons were discarded) using PandaSeq, demultiplexed and mapped against the GREENGENES reference database (v13.8)². Sequences with <60% similarity to the reference were removed. Remaining sequences were clustered with Usearch61 at 97% sequence similarity against the GREENGENES database using a closed-reference picking process, and taxonomic assignment was done using the RDP

classifier³. After taxonomic assignment, operational taxonomic units (OTUs) representing bacterial origin were selected, and bacterial OTUs with overall relative abundance below 0.01% were excluded from downstream analyses. Data were rarefied to 13,000 sequences per sample to adjust the various sequencing depths among samples. With the recommended pipeline in QIIME, relative abundance of bacterial OTUs was summarized at the phylum to genus levels. Microbial alpha diversity within samples was calculated as Chao1 richness, observed OTUs, phylogenetic diversity (PD) whole tree index, Simpson index and Shannon index. These diversity indices were presented for the whole microbiota as well as under each specific phylum of four dominant phyla (Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria) in infant gut. Microbial community differences between samples (beta diversity) were measured as Jensen-Shannon distance between the genus-level profiles. Metagenomic functions were predicted based on the closed-reference picked OTU table using the PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) pipeline⁴. Human disease and organismal system-related functions were removed, and the abundances of level-3 functions were normalized for downstream analyses.

qPCR for *Clostridioides difficile* (*C. difficile*) Detection

Detailed protocol for detection of *C. difficile* colonization was stated in our recent study⁵. A 16S primer pair and a probe set targeted to 16S rRNA gene of *C. difficile* were used as described by Penders et al⁶. To minimize differential inhibitory effects due to variable concentrations of template genomic DNA in qPCR, all DNA samples were first normalized by dilution to 1 ng/ μ L⁷. Consequently, each multiplex assay was prepared to contain 1X QuantiNova Multiplex PCR Kit (QIAGEN), 0.4 μ M of each primer, 0.25 μ M of each probe and 1 μ L [1 ng/ μ L] of sample DNA in a final volume of 20 μ L. qPCR cycling conditions were as follows: initial denaturation for 2min at 95.0°C, 40 cycles of denaturation

for 5s at 95°C and annealing/extension/reading for 20s at 60°C. A standard curve was created and employed to determine the efficiency of the *C. difficile* primers and probes by performing five 1:10 serial dilutions of *C. difficile* ATCC 9689D-5 genomic DNA starting at 1 ng/μL. We calculated the lower limit of detection for the multiplex assay to be 1X10⁻⁵ ng of DNA or 2 genomes of *C. difficile* based on the amplification data from the serial dilution and the non-template control. Since different starting mass of fecal sample generated each template DNA, the limit of quantification for the analysis was different from sample-to-sample. In this study, the quantitative results of *C. difficile* was transformed to binary variable (colonized or not colonized).

Statistical Analysis

To illustrate the compositional differences in the microbial populations, all samples were clustered using the partitioning around medoids (PAM) clustering algorithm previously described⁸, which clustered fecal microbiota samples by their weighted UniFrac distances and abundances into account in the computational model. The optimal number of clusters was determined by the Calinski-Harabasz index and Silhouette width. Four developmental trajectories of infant gut microbiota were determined according to the shift of cluster membership. The linear discriminant analysis effect size (LEfSe)⁹ was used to determine both taxonomic and function biomarkers and the results are plotted with a log score cut-off of 3 (for bacterial genera) and 2 (for microbiota functions) to identify differentially abundant bacterial phyla and genera, and predicted functions between groups. Comparisons of beta-diversity between different groups were done using Wilcoxon's tests. Comparisons of richness and diversity indices between two groups and more than three groups were made using Wilcoxon's tests and Dunn's tests paired with Benjamini-Hochberg correction, respectively. Bivariate analyses were used to detect potential associations between covariates

and developmental trajectories, as well as between covariates and outcomes (Table S2-8). In details, Fisher's exact tests were used for categorical data; *t*-tests/analysis of variance (ANOVA) and Wilcoxon's tests/Kruskal-Wallis tests were performed for two/four-group comparisons of continuous parameters that were and were not normally distributed, respectively. Canonical correspondence analysis (CCA) was used to study the relationship between exposures and the early and late infancy gut microbiota community structures. Age at sampling, location, birth modes, feeding status in early infancy, direct antibiotic exposure during the first 3 months, presence and absence of older siblings, and pet exposure (pre- and postnatal), sex, gravida, gestational age, maternal age, maternal race, maternal obesity, maternal diabetes, maternal atopy, maternal food allergy, and maternal asthma were included in the analysis for early infancy samples, while for late infancy samples, antibiotic exposure during the first 3 months was replaced by antibiotic exposure during the first year, and solid food introduction was further added. Only those showing significant associations with cluster memberships in bivariate analyses were shown in the figures.

Binary logistic regression models were used to quantify the associations between atopy outcomes and microbiota features (developmental trajectories and colonization of *C. difficile*) or trajectories combined with ethnicity or *C. difficile*, while adjusting for other covariates. Three models were tested for each association: Model 1, a crude model tested for each microbiota feature as the independent variable. Model 2 contained sampling time and five known influential factors in addition to Model 1. On top of Model 2, Model 3 further added potential confounders based on the directed acyclic graph (DAG) of the association between microbial developmental trajectories and atopy (Figure S4). DAG was illustrated using the R package "dagitty"¹⁰.

A *post hoc* nested case-control analysis using mixed models optimized for zero-inflated microbiome data¹¹ was performed to detect differentially abundant taxa (prevalent in at least 20% samples in each time point) between sensitized and non-sensitized infants.

Effect modifications were assessed in the multiplicative scale and additive scale. Additive scale modifications were measured by relative excessive risk due to interaction while the confidence intervals were generated using 1000 bootstraps. Mediation analysis was conducted using the R package “mediation”, while confidence interval and p values were generated using 1000 bootstraps.

For comparisons of metabolites and sIgA between trajectories, observations of C2-C1 samples were removed due to the low sample size (metabolites: 4 early-infancy samples, 2 late-infancy samples; sIgA: 6 early-infancy samples, 2 late-infancy samples). Concentrations were converted into age-adjusted zscores. Comparisons were done using *t* tests paired with Benjamini-Hochberg correction.

Pairwise deletion was performed in bivariate analysis, while listwise deletion was performed in modeling for missing data. Statistical significance was set at $\alpha=0.05$.

Supplementary Figures

Figure S1. Phylum-level relative abundances at different chronological ages of sampling.

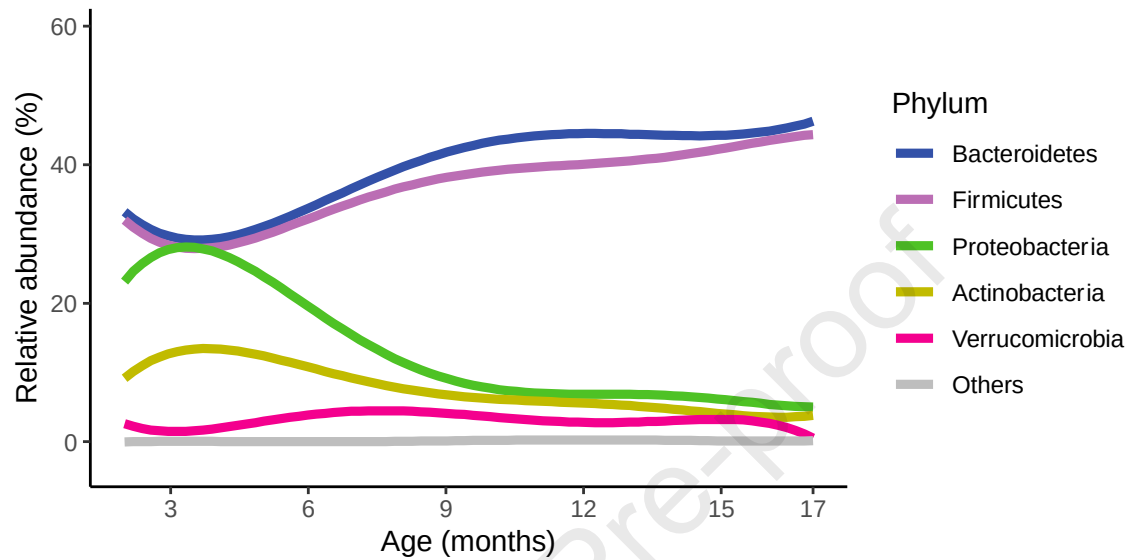


Figure S2. Calinski-Harabasz index and average Silhouette width peaks at k=2 in PAM clustering. (A) Calinski-Harabasz index. (B) average Silhouette width.

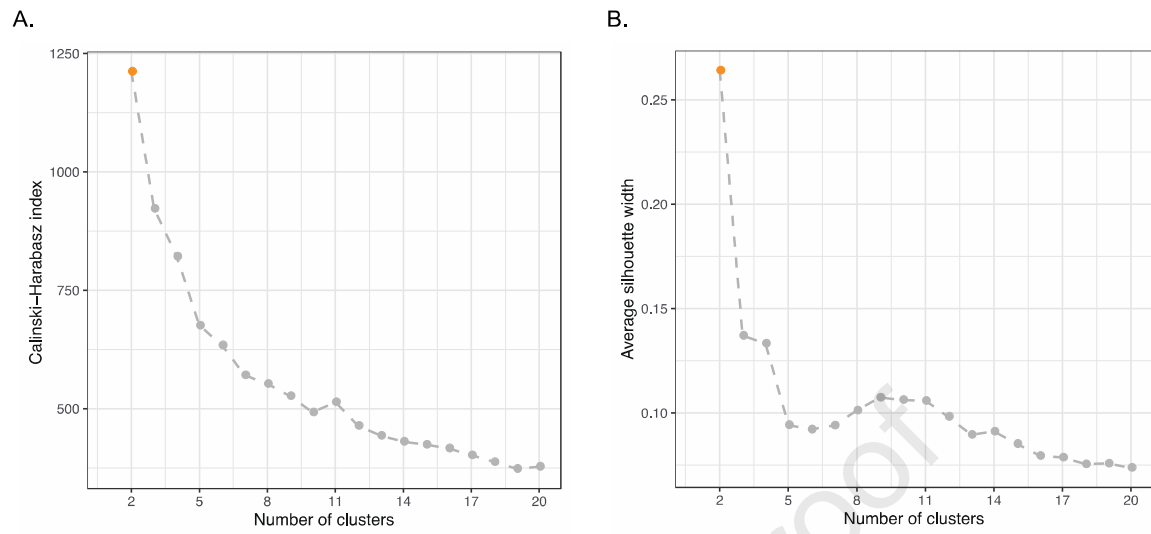


Figure S3. Distributions of E/B ratios by trajectories. (A) Family-level E/B ratio. (B) Genus-level. P values were given by Dunn's tests, corrected by the Benjamini-Hochberg method. Only p values for four comparisons are shown: between C1-C1 and the other three trajectories, and between trajectory C1-C2 and C2-C2.

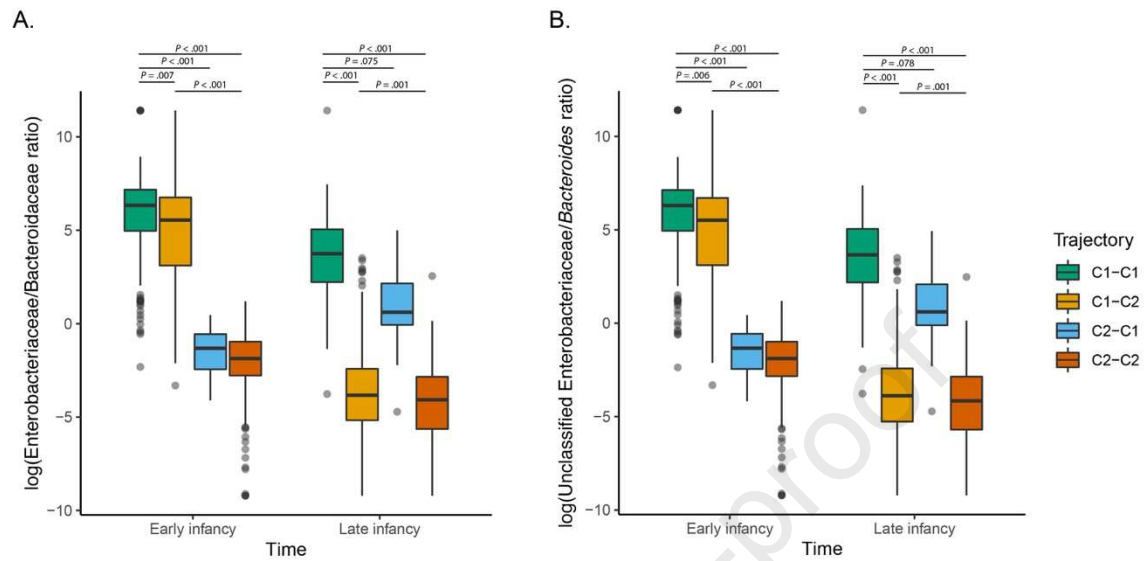


Figure S4. Directed acyclic graph (DAG) of the association between the microbiota development and atopy. CS: C-section; Mat atopy: maternal atopy; BF: breastfeeding; abx: antibiotic exposure; solids: solid food introduction in age 3 months.

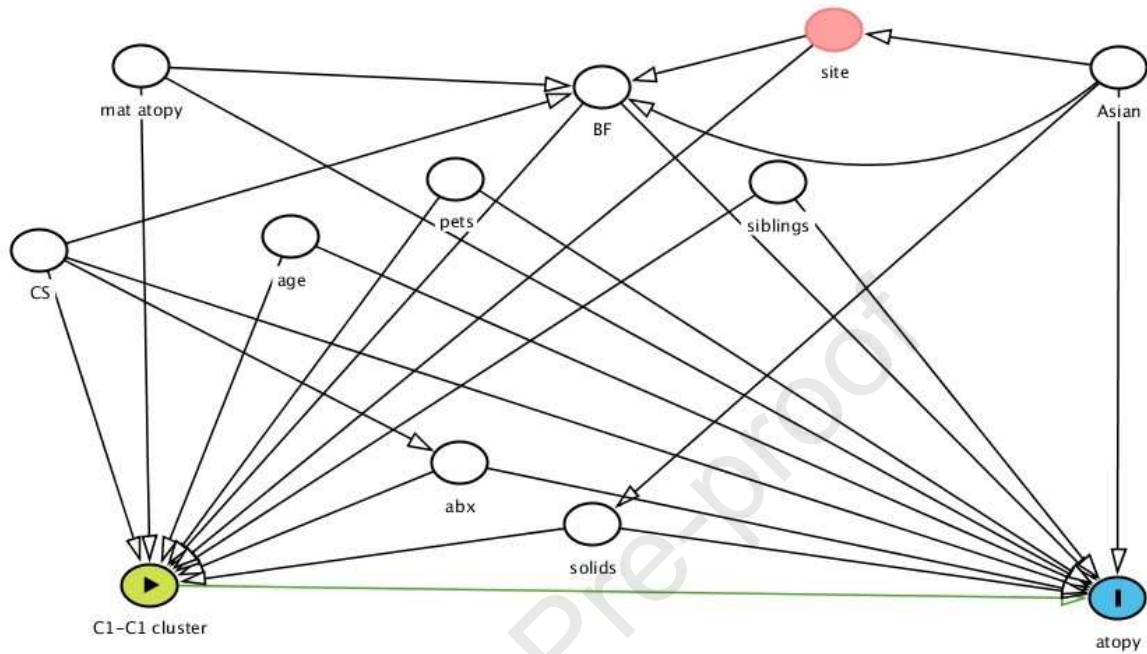


Figure S5. Associations of food sensitization with microbiota developmental trajectories in the first year, stratified by ethnicity. (A) Association between food sensitization at age 3 years and microbiota developmental trajectories. (B) Association between peanut sensitization at age 3 years and microbiota developmental trajectories. Odds ratios were presented with 95% confidence intervals. Those that were statistically significant were shown in orange. The vertical dashed lines were located at OR = 1 for reference.

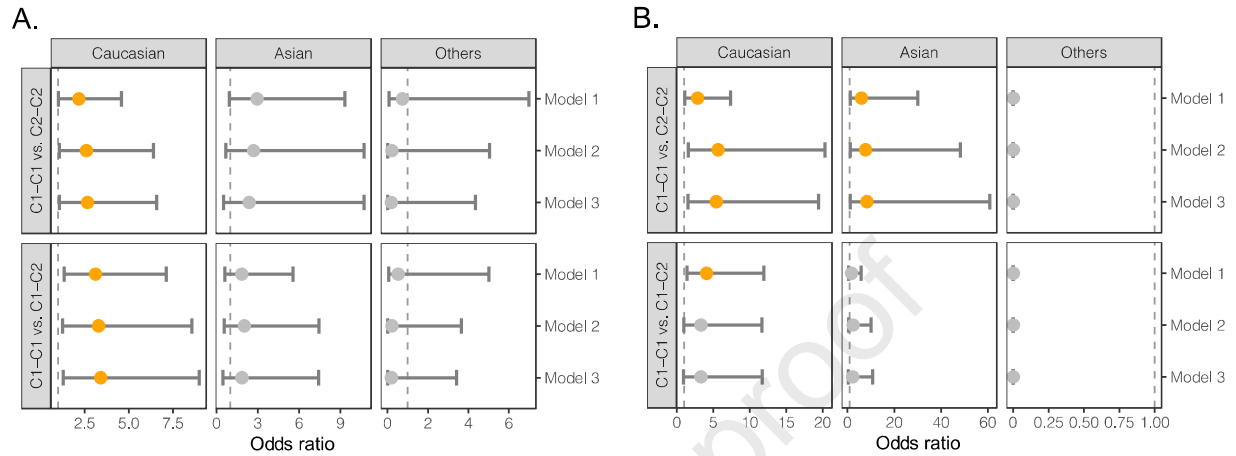


Figure S6. Predicted microbiota functions enriched under different developmental trajectories in (A) early or (B) late-infancy.

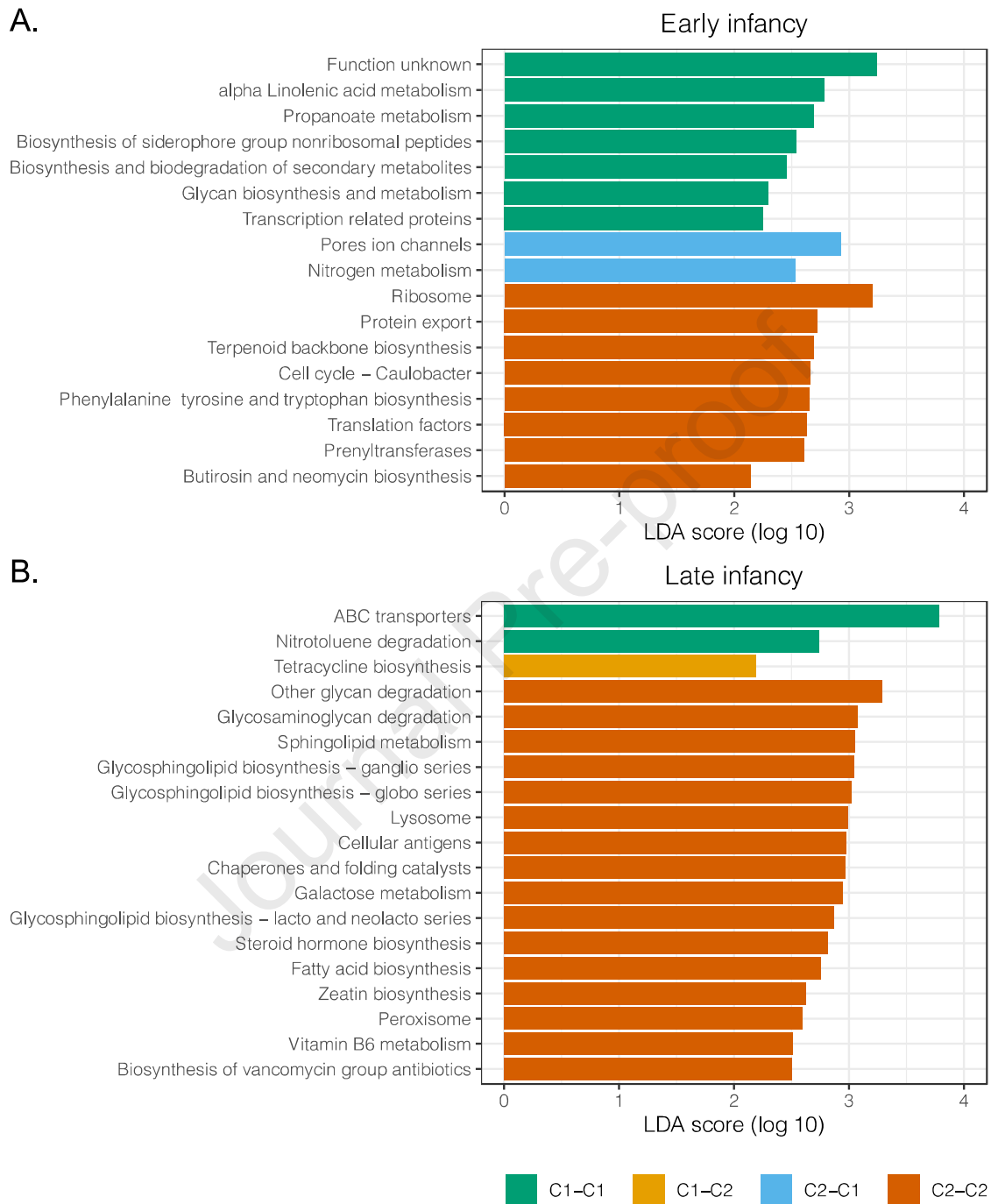


Figure S7. Abundances of predicted sphingolipid metabolism under different trajectories influenced by maternal ethnicity in early or late-infancy. Comparisons of the abundances were made for all samples and by developmental trajectories using Dunn's tests paired with Benjamini-Hochberg correction.

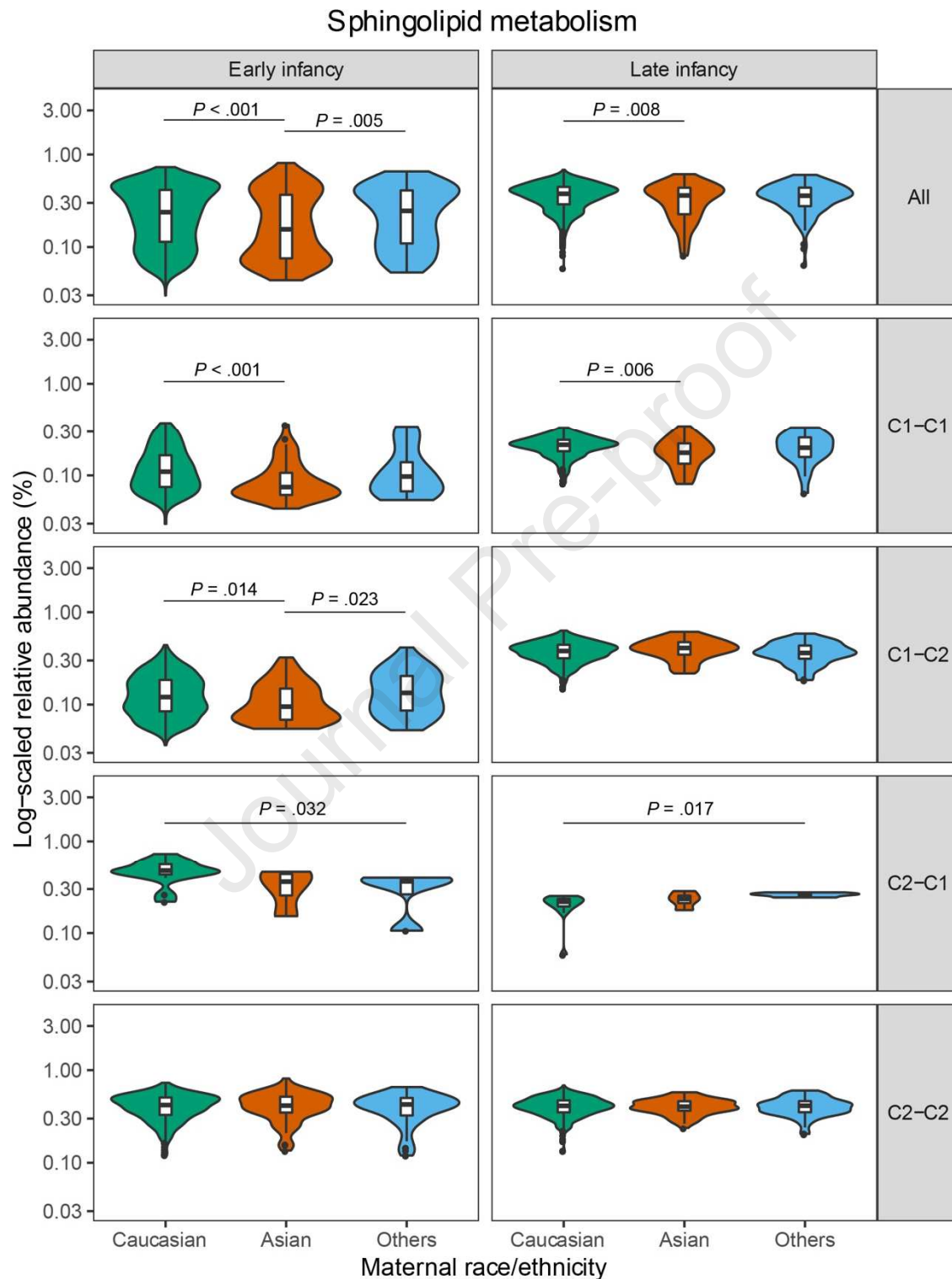


Figure S8. Abundances of predicted glycosphingolipid biosynthesis (ganglio series) under different trajectories influenced by maternal ethnicity in early or late-infancy. Comparisons of the abundances were made for all samples and by developmental trajectories using Dunn's tests paired with Benjamini-Hochberg correction.

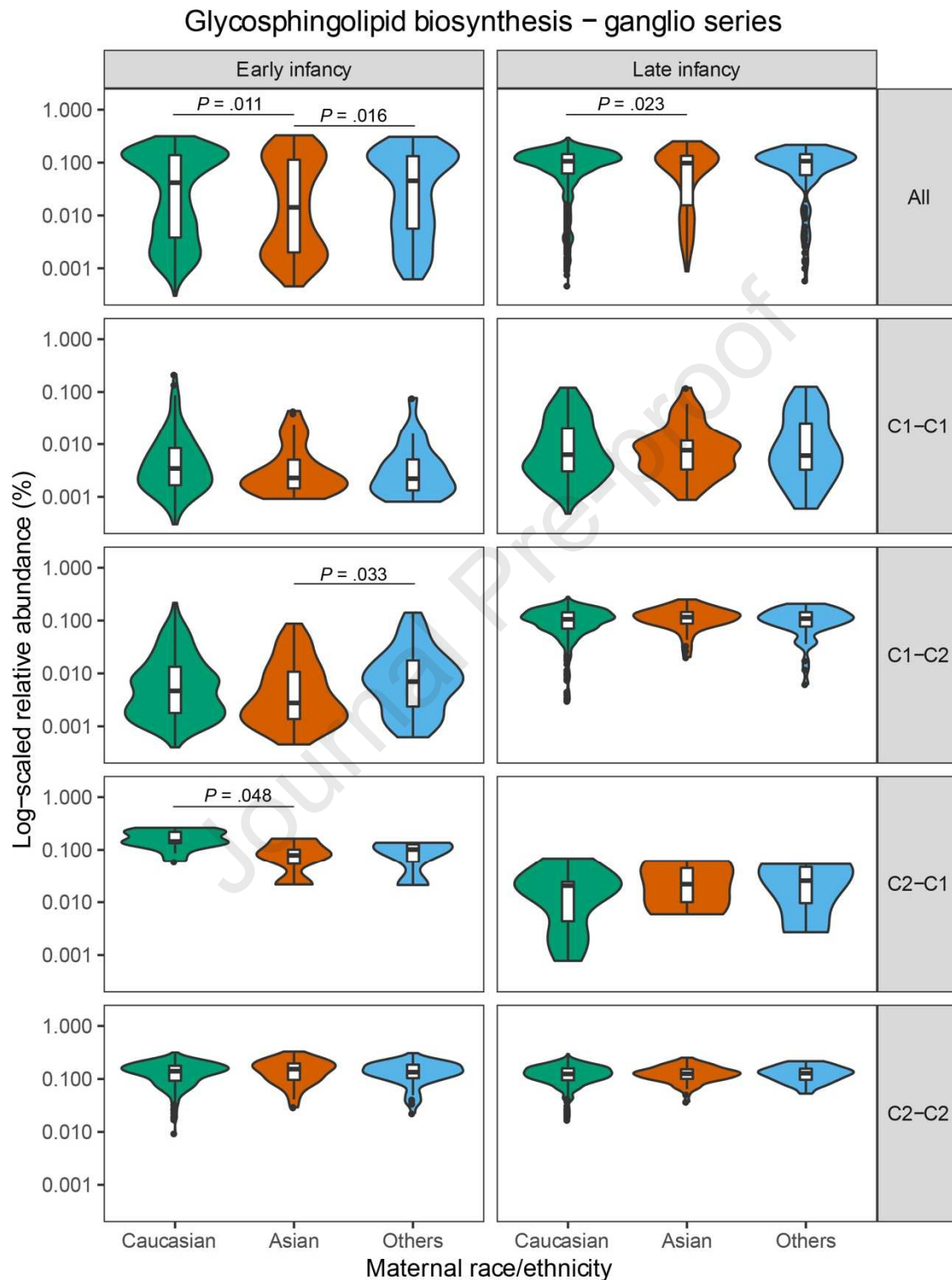


Figure S9. Abundances of predicted glycosphingolipid biosynthesis (globo series) under different trajectories influenced by maternal ethnicity in early or late-infancy.
Comparisons of the abundances were made for all samples and by developmental trajectories using Dunn's tests paired with Benjamini-Hochberg correction.

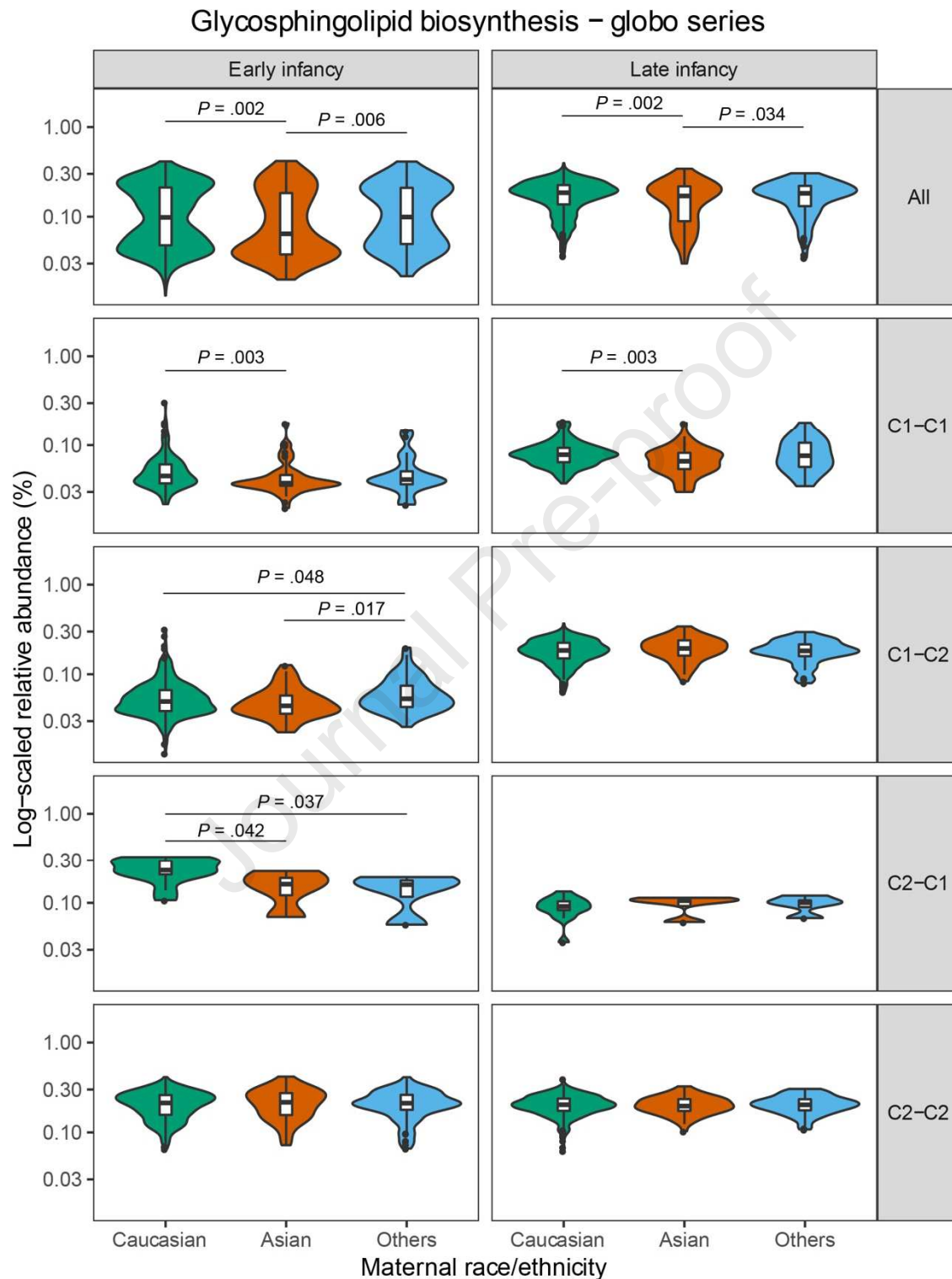


Figure S10. Abundances of predicted glycosphingolipid biosynthesis (lacto and neolacto series) under different trajectories influenced by maternal ethnicity in early or late-infancy. Comparisons of the abundances were made for all samples and by developmental trajectories using Dunn's tests paired with Benjamini-Hochberg correction.

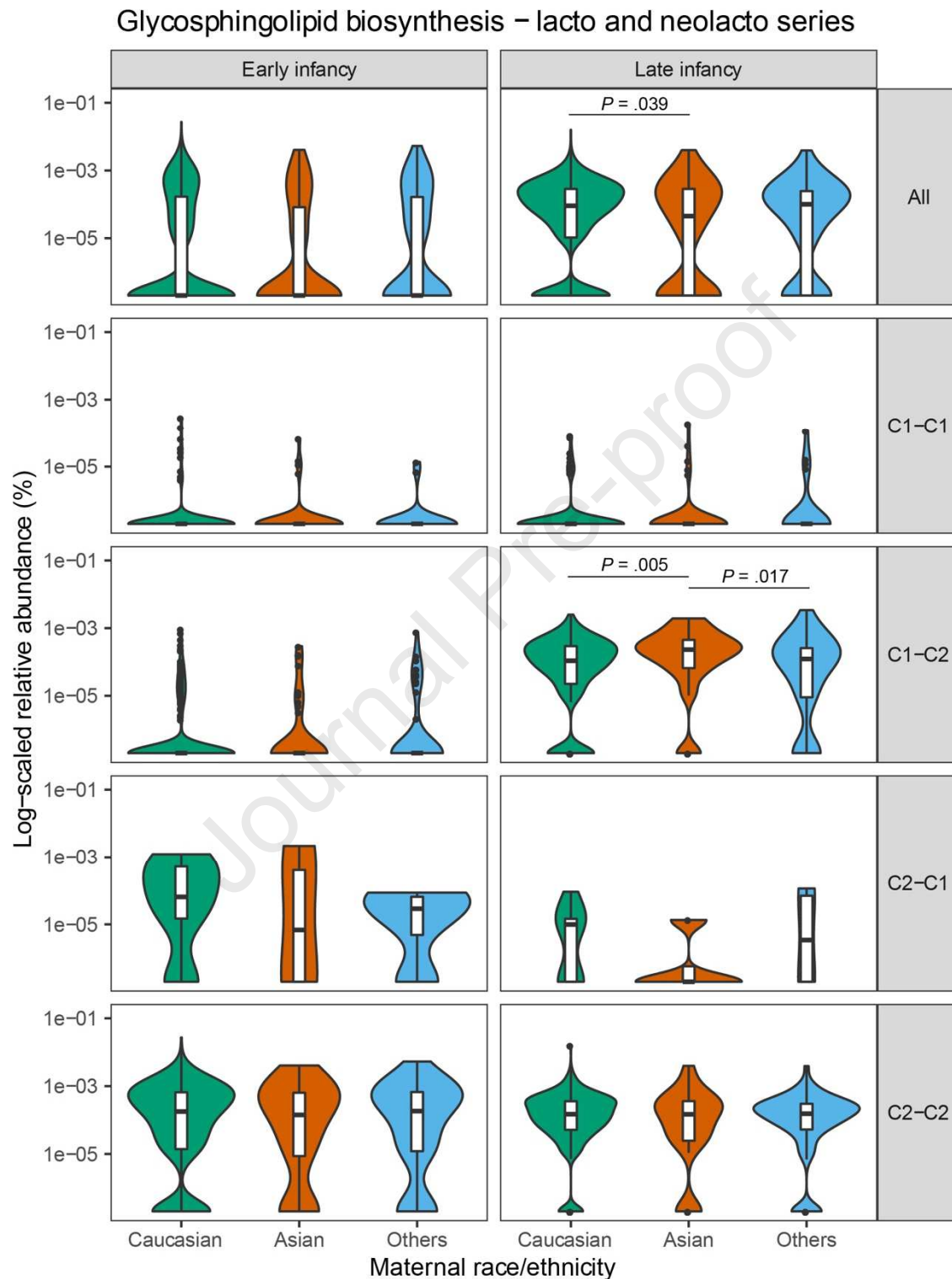
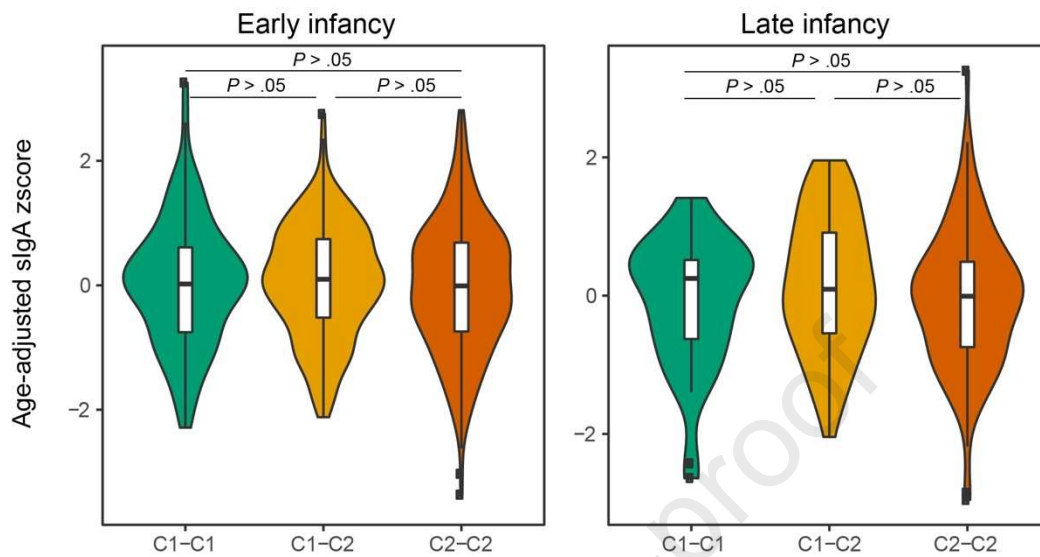


Figure S11. Age-adjusted secretory IgA zscores by developmental trajectories in early and late-infancy microbiota. C2-C1 samples were removed from this analysis due to small sample sizes (6 for early-infancy samples and 3 for late-infancy samples). Comparisons of the zscores were made using *t* tests paired with Benjamini-Hochberg correction.



Supplementary Tables

Table S1. Characteristics of participants in the general CHILd cohort and in the current study.

	Overall CHILd Population (N= 3264)	Current Study (N=1422)	<i>p</i> value
Maternal age	32.4 (29.3, 35.5)	32.3 (29.3, 35.3)	0.602
Gestation age (week)	39 (38, 40)	39 (38, 40)	0.025
Gravida	2 (1, 3)	2 (1, 3)	0.650
Location*			<0.001
Vancouver	730 (22.4)	471 (33.1)	
Edmonton	765 (23.4)	308 (21.7)	
Winnipeg	1004 (30.8)	643 (45.2)	
Toronto	765 (23.4)	0 (0) [#]	
Maternal prepregnancy weight status			0.187
Normal	1880 (63.3)	835 (61.2)	
Overweight or obese	1089 (36.7)	529 (38.8)	
Maternal prenatal diabetes			0.517
No	3054 (94.7)	1344 (95.2)	
Yes	171 (5.3)	68 (4.8)	
Maternal prenatal atopy			0.261
No	538 (17.1)	218 (15.7)	
Yes	2616 (82.9)	1171 (84.3)	
Maternal prenatal asthma			1
No	2901 (91.9)	1304 (91.9)	
Yes	255 (8.1)	115 (8.1)	
Maternal ethnicity			0.003
Caucasian	2338 (72.8)	1091 (77.5)	
Asian	452 (14.1)	171 (12.1)	
Others	420 (13.1)	146 (10.4)	
Sex			0.775
Female	1546 (47.4)	667 (46.9)	
Male	1717 (52.6)	755 (53.1)	
Birth mode			0.609
Vaginal, IAP-	1694 (53.1)	741 (53.5)	
Vaginal, IAP+	687 (21.5)	313 (22.6)	
Cesarean, scheduled	346 (10.8)	134 (9.7)	
Cesarean, emergency	465 (14.6)	198 (14.3)	
Breastfeeding status (0-3 mo)			0.29
Formula fed	446 (14.2)	226 (15.9)	
Partially breast fed	821 (26.1)	369 (25.9)	
Exclusively breast fed	1884 (59.8)	827 (58.2)	
Direct antibiotic exposure (0-12 mo)			0.032
No	2536 (82.6)	980 (79.7)	
Yes	535 (17.4)	249 (20.3)	

Siblings			0.058
No	1664 (51.8)	676 (48.7)	
Yes	1548 (48.2)	711 (51.3)	
Solid food introduction (0-3 mo)			0.163
No	2768 (91.9)	1300 (93.1)	
Yes	245 (8.1)	96 (6.9)	
Household pet exposure			0.936
No	1441 (50.4)	707 (49.8)	
Only Prenatal	199 (7.0)	95 (6.7)	
Both Pre- and Postnatal	1185 (41.4)	603 (42.4)	
Only Postnatal	34 (1.2)	16 (1.1)	

Continuous data were shown in median (1st quartile, 3rd quartile), and differences were evaluated using Wilcoxon rank-sum tests. Categorical data were shown in n (% proportion), and differences were evaluated using Fisher's exact tests.

* Samples from Toronto site were excluded since provincial prescription database for assessing antibiotic medication was not available.

Table S2. Key characteristics of infants by trajectory and maternal ethnicity.

		Asian	Caucasian	Others	<i>p</i> value
All infants	Birth mode				0.458
	Vaginal, IAP-	85 (50)	578 (54.4)	68 (48.2)	
	Vaginal, IAP+	37 (21.8)	235 (22.1)	39 (27.7)	
	Cesarean, scheduled	21 (12.4)	101 (9.5)	11 (7.8)	
	Cesarean, emergency	27 (15.9)	148 (13.9)	23 (16.3)	
	Breastfeeding status (0-3 months)				0.374
	Formula fed	23 (13.5)	173 (15.9)	27 (18.5)	
	Partially breastfed	48 (28.1)	274 (25.1)	44 (30.1)	
	Exclusively breastfed	100 (58.5)	644 (59)	75 (51.4)	
	Siblings				0.89
	No	86 (12.8)	516 (76.8)	70 (10.4)	
	Yes	84 (12)	546 (77.8)	72 (10.3)	
	Solid food introduction (0-3 months)				0.005
	No	165 (98.2)	995 (92.9)	130 (90.3)	
	Yes	3 (1.8)	76 (7.1)	14 (9.7)	
	<i>C. difficile</i> colonization (absent (A) and present (P) colonization at early and late infancy)				<0.001
	A-P	69 (44.8)	261 (26.5)	24 (18.8)	
	P-P	22 (14.3)	140 (14.2)	20 (15.6)	
	P-A	5 (3.2)	175 (17.8)	20 (15.6)	
	A-A	58 (37.7)	409 (41.5)	64 (50)	
C2-C2 infants	Birth mode				0.3
	Vaginal, IAP-	45 (71.4)	347 (70.8)	3 (59.1)	
	Vaginal, IAP+	12 (19)	106 (21.6)	20 (30.3)	
	Cesarean, scheduled	3 (4.8)	14 (2.9)	1 (1.5)	
	Cesarean, emergency	3 (4.8)	23 (4.7)	6 (9.1)	
	Breastfeeding status (0-3 months)				0.214
	Formula fed	8 (12.5)	89 (17.6)	18 (26.5)	
	Partially breastfed	20 (31.2)	142 (28)	21 (30.9)	
	Exclusively breastfed	36 (56.2)	276 (54.4)	29 (42.6)	
	Siblings				0.392
	No	29 (10.7)	218 (80.4)	24 (8.9)	
	Yes	34 (9.6)	277 (78.2)	43 (12.1)	
	Solid food introduction (0-3 months)				0.018
	No	64 (100)	456 (92.9)	60 (89.6)	
	Yes	0 (0)	35 (7.1)	7 (10.4)	
	<i>C. difficile</i> colonization (absent (A) and present (P) colonization at early and late infancy)				0.004
	A-P	26 (41.9)	140 (28.7)	13 (20.3)	
	P-P	9 (14.5)	68 (14)	7 (10.9)	
	P-A	1 (1.6)	79 (16.2)	8 (12.5)	
	A-A	26 (41.9)	200 (41.1)	36 (56.2)	

C1-C2 infants	Birth mode				0.912
	Vaginal, IAP-	22 (42.3)	168 (40.4)	19 (39.6)	
	Vaginal, IAP+	12 (23.1)	89 (21.4)	12 (25)	
	Cesarean, scheduled	11 (21.2)	75 (18)	7 (14.6)	
	Cesarean, emergency	7 (13.5)	84 (20.2)	10 (20.8)	
	Breastfeeding status (0-3 months)				0.307
	Formula fed	10 (19.2)	65 (15.4)	5 (9.8)	
	Partially breastfed	16 (30.8)	98 (23.2)	16 (31.4)	
	Exclusively breastfed	26 (50)	260 (61.5)	3 (58.8)	
	Siblings				0.296
	No	24 (9.8)	192 (78.7)	28 (11.5)	
	Yes	28 (10.4)	221 (82.2)	20 (7.4)	
	Solid food introduction (0-3 months)				0.903
	No	48 (94.1)	388 (92.6)	46 (92)	
	Yes	3 (5.9)	31 (7.4)	4 (8)	
	C. difficile colonization (absent (A) and present (P) colonization at early and late infancy)				0.004
	A-P	21 (45.7)	86 (24.2)	6 (14)	
	P-P	7 (15.2)	42 (11.8)	10 (23.3)	
	P-A	3 (6.5)	73 (20.5)	9 (20.9)	
	A-A	15 (32.6)	155 (43.5)	18 (41.9)	
C1-C1 infants	Birth mode				0.992
	Vaginal, IAP-	17 (33.3)	55 (38.7)	8 (34.8)	
	Vaginal, IAP+	12 (23.5)	34 (23.9)	6 (26.1)	
	Cesarean, scheduled	5 (9.8)	12 (8.5)	2 (8.7)	
	Cesarean, emergency	17 (33.3)	41 (28.9)	7 (30.4)	
	Breastfeeding status (0-3 months)				0.797
	Formula fed	4 (7.8)	19 (12.9)	3 (13)	
	Partially breastfed	10 (19.6)	30 (20.4)	6 (26.1)	
	Exclusively breastfed	37 (72.5)	98 (66.7)	14 (60.9)	
	Siblings				0.535
	No	32 (21.9)	99 (67.8)	15 (10.3)	
	Yes	19 (27.9)	41 (60.3)	8 (11.8)	
	Solid food introduction (0-3 months)				0.203
	No	49 (100)	138 (93.9)	22 (95.7)	
	Yes	0 (0)	9 (6.1)	1 (4.3)	
	C. difficile colonization (absent (A) and present (P) colonization at early and late infancy)				0.037
	A-P	21 (50)	34 (26.6)	5 (29.4)	
	P-P	5 (11.9)	29 (22.7)	2 (11.8)	
	P-A	1 (2.4)	20 (15.6)	3 (17.6)	
	A-A	15 (35.7)	45 (35.2)	7 (41.2)	

Continuous data were shown in median (1st quartile, 3rd quartile), and differences were evaluated using Kruskal-Wallis tests. Categorical data were shown in n (% proportion), and differences were evaluated using Fisher's exact tests.

Table S3. Population characteristics stratified by atopic sensitization at age 1 year.

	Atopic sensitization at age 1 year		<i>p</i> value
	No	Yes	
Gestation age (week)	39 (38, 40)	39 (38, 40)	0.761
Location			<0.001
Vancouver	390 (31.4)	81 (47.9)	
Edmonton	265 (21.3)	40 (23.7)	
Winnipeg	589 (47.3)	48 (28.4)	
Maternal prepregnancy weight status			0.225
Normal	728 (60.8)	105 (66)	
Overweight or obese	469 (39.2)	54 (34)	
Maternal prenatal diabetes			0.435
No	1179 (95.5)	158 (94)	
Yes	56 (4.5)	10 (6)	
Maternal prenatal atopy			0.910
No	192 (15.8)	25 (15.1)	
Yes	1022 (84.2)	141 (84.9)	
Maternal prenatal food allergy			0.039
No	925 (74.9)	113 (67.3)	
Yes	310 (25.1)	55 (32.7)	
Maternal prenatal asthma			0.649
No	1144 (92.1)	153 (91.1)	
Yes	98 (7.9)	15 (8.9)	
Maternal ethnicity			<0.001
Caucasian	977 (79.4)	108 (64.3)	
Asian	124 (10.1)	46 (27.4)	
Others	130 (10.6)	14 (8.3)	
Sex			1.000
Female	579 (46.5)	79 (46.7)	
Male	665 (53.5)	90 (53.3)	
Birth mode			0.870
Vaginally born without IAP	651 (53.5)	86 (53.4)	
Vaginally born with IAP	276 (22.7)	33 (20.5)	
Scheduled C-section	116 (9.5)	17 (10.6)	
Emergency C-section	173 (14.2)	25 (15.5)	
Breastfeeding status (0-3 mo)			0.626
Formula fed	200 (16.1)	23 (13.6)	
Partially breast fed	326 (26.2)	42 (24.9)	
Exclusively breast fed	718 (57.7)	104 (61.5)	
Direct antibiotic exposure (0-12 mo)			0.326
No	851 (79.3)	123 (83.1)	
Yes	222 (20.7)	25 (16.9)	
Siblings			0.362

No	584 (48.1)	86 (52.1)	
Yes	629 (51.9)	79 (47.9)	
Solid food introduction (0-3 mo)			0.007
No	1135 (92.6)	158 (98.1)	
Yes	91 (7.4)	3 (1.9)	
Household pet exposure			0.024
No	598 (48.1)	102 (60.4)	
Only Prenatal	87 (7)	8 (4.7)	
Both Pre- and Postnatal	544 (43.8)	57 (33.7)	
Only Postnatal	14 (1.1)	2 (1.2)	
Trajectory			0.014
C1-C1	177 (14.2)	40 (23.7)	
C1-C2	465 (37.4)	61 (36.1)	
C2-C1	21 (1.7)	1 (0.6)	
C2-C2	581 (46.7)	67 (39.6)	
<i>C. difficile</i> colonization (early to late infancy)			0.676
A-P	312 (27.8)	45 (30)	
P-P	165 (14.7)	18 (12)	
P-A	175 (15.6)	27 (18)	
A-A	472 (42)	60 (40)	

Continuous data were shown in median (1st quartile, 3rd quartile), and differences were evaluated using Wilcoxon rank-sum tests. Categorical data were shown in n (% proportion), and differences were evaluated using Fisher's exact tests.

Table S4. Population characteristics stratified by atopic sensitization at age 3 years.

	Atopic sensitization at age 3 years		<i>p</i> value
	No	Yes	
Gestation age (week)	39 (38, 40)	39 (38, 40)	0.092
Location			<0.001
Vancouver	351 (31.2)	77 (46.4)	
Edmonton	223 (19.8)	42 (25.3)	
Winnipeg	552 (49)	47 (28.3)	
Maternal prepregnancy weight status			0.053
Normal	666 (61.4)	110 (69.6)	
Overweight or obese	418 (38.6)	48 (30.4)	
Maternal prenatal diabetes			0.313
No	1073 (95.7)	155 (93.9)	
Yes	48 (4.3)	10 (6.1)	
Maternal prenatal atopy			0.907
No	171 (15.5)	24 (14.7)	
Yes	931 (84.5)	139 (85.3)	
Maternal prenatal food allergy			0.507
No	831 (74.1)	118 (71.5)	
Yes	290 (25.9)	47 (28.5)	
Maternal prenatal asthma			0.645
No	1035 (92.1)	150 (90.9)	
Yes	89 (7.9)	15 (9.1)	
Maternal ethnicity			<0.001
Caucasian	893 (79.9)	96 (58.2)	
Asian	111 (9.9)	49 (29.7)	
Others	114 (10.2)	20 (12.1)	
Sex			<0.001
Female	549 (48.8)	54 (32.5)	
Male	577 (51.2)	112 (67.5)	
Birth mode			0.246
Vaginally born without IAP	584 (53.1)	88 (54.7)	
Vaginally born with IAP	261 (23.7)	28 (17.4)	
Scheduled C-section	103 (9.4)	18 (11.2)	
Emergency C-section	151 (13.7)	27 (16.8)	
Breastfeeding status (0-3 mo)			0.899
Formula fed	175 (15.5)	27 (16.3)	
Partially breast fed	287 (25.5)	44 (26.5)	
Exclusively breast fed	664 (59)	95 (57.2)	
Direct antibiotic exposure (0-12 mo)			0.580
No	783 (80.1)	114 (78.1)	
Yes	194 (19.9)	32 (21.9)	
Siblings			0.452

No	532 (48.5)	85 (51.8)	
Yes	564 (51.5)	79 (48.2)	
Solid food introduction (0-3 mo)			0.618
No	1027 (92.9)	155 (94.5)	
Yes	78 (7.1)	9 (5.5)	
Household pet exposure			0.008
No	536 (47.6)	100 (60.2)	
Only Prenatal	81 (7.2)	8 (4.8)	
Both Pre- and Postnatal	498 (44.3)	55 (33.1)	
Only Postnatal	10 (0.9)	3 (1.8)	
Trajectory			0.019
C1-C1	162 (14.4)	39 (23.5)	
C1-C2	433 (38.5)	51 (30.7)	
C2-C1	18 (1.6)	3 (1.8)	
C2-C2	513 (45.6)	73 (44)	
<i>C. difficile</i> colonization (early to late infancy)			0.596
A-P	281 (27.7)	46 (30.7)	
P-P	144 (14.2)	25 (16.7)	
P-A	157 (15.5)	23 (15.3)	
A-A	431 (42.5)	56 (37.3)	

Continuous data were shown in median (1st quartile, 3rd quartile), and differences were evaluated using Wilcoxon rank-sum tests. Categorical data were shown in n (% proportion), and differences were evaluated using Fisher's exact tests.

Table S5. Population characteristics stratified by food sensitization at age 1 year.

	Food sensitization at age 1 year		<i>p</i> value
	No	Yes	
Gestation age (week)	39 (38, 40)	40 (38, 40)	0.765
Location			0.002
Vancouver	411 (32.1)	60 (44.8)	
Edmonton	273 (21.3)	32 (23.9)	
Winnipeg	595 (46.5)	42 (31.3)	
Maternal prepregnancy weight status			0.340
Normal	749 (61)	84 (65.6)	
Overweight or obese	479 (39)	44 (34.4)	
Maternal prenatal diabetes			0.127
No	1214 (95.6)	123 (92.5)	
Yes	56 (4.4)	10 (7.5)	
Maternal prenatal atopy			0.706
No	195 (15.6)	22 (16.8)	
Yes	1054 (84.4)	109 (83.2)	
Maternal prenatal food allergy			0.037
No	950 (74.8)	88 (66.2)	
Yes	320 (25.2)	45 (33.8)	
Maternal prenatal asthma			0.737
No	1173 (91.9)	124 (93.2)	
Yes	104 (8.1)	9 (6.8)	
Maternal ethnicity			<0.001
Caucasian	998 (78.9)	87 (64.9)	
Asian	134 (10.6)	36 (26.9)	
Others	133 (10.5)	11 (8.2)	
Sex			0.650
Female	593 (46.4)	65 (48.5)	
Male	686 (53.6)	69 (51.5)	
Birth mode			0.958
Vaginally born without IAP	666 (53.3)	71 (55.5)	
Vaginally born with IAP	282 (22.6)	27 (21.1)	
Scheduled C-section	122 (9.8)	11 (8.6)	
Emergency C-section	179 (14.3)	19 (14.8)	
Breastfeeding status (0-3 mo)			0.852
Formula fed	204 (15.9)	19 (14.2)	
Partially breast fed	334 (26.1)	34 (25.4)	
Exclusively breast fed	741 (57.9)	81 (60.4)	
Direct antibiotic exposure (0-12 mo)			0.279
No	874 (79.3)	100 (84)	
Yes	228 (20.7)	19 (16)	
Siblings			0.170

No	598 (48)	72 (54.5)	
Yes	648 (52)	60 (45.5)	
Solid food introduction (0-3 mo)			0.009
No	1168 (92.7)	125 (98.4)	
Yes	92 (7.3)	2 (1.6)	
Household pet exposure			0.015
No	617 (48.3)	83 (61.9)	
Only Prenatal	87 (6.8)	8 (6)	
Both Pre- and Postnatal	560 (43.8)	41 (30.6)	
Only Postnatal	14 (1.1)	2 (1.5)	
Trajectory			0.006
C1-C1	183 (14.3)	34 (25.4)	
C1-C2	479 (37.5)	47 (35.1)	
C2-C1	22 (1.7)	0 (0)	
C2-C2	595 (46.5)	53 (39.6)	
<i>C. difficile</i> colonization (early to late infancy)			0.774
A-P	325 (28.2)	32 (26.7)	
P-P	167 (14.5)	16 (13.3)	
P-A	179 (15.5)	23 (19.2)	
A-A	483 (41.9)	49 (40.8)	

Continuous data were shown in median (1st quartile, 3rd quartile), and differences were evaluated using Wilcoxon rank-sum tests. Categorical data were shown in n (% proportion), and differences were evaluated using Fisher's exact tests.

Table S6. Population characteristics stratified by food sensitization at age 3 years.

	Food sensitization at age 3 years		<i>p</i> value
	No	Yes	
Gestation age (week)	39 (38, 40)	39 (38, 40)	0.185
Location			0.059
Vancouver	396 (32.6)	31 (41.3)	
Edmonton	246 (20.2)	19 (25.3)	
Winnipeg	573 (47.2)	25 (33.3)	
Maternal prepregnancy weight status			0.898
Normal	731 (62.4)	44 (63.8)	
Overweight or obese	441 (37.6)	25 (36.2)	
Maternal prenatal diabetes			0.769
No	1154 (95.5)	73 (97.3)	
Yes	55 (4.5)	2 (2.7)	
Maternal prenatal atopy			1.000
No	184 (15.5)	11 (14.9)	
Yes	1005 (84.5)	63 (85.1)	
Maternal prenatal food allergy			0.503
No	889 (73.5)	58 (77.3)	
Yes	320 (26.5)	17 (22.7)	
Maternal prenatal asthma			0.121
No	1118 (92.2)	65 (86.7)	
Yes	94 (7.8)	10 (13.3)	
Maternal ethnicity			<0.001
Caucasian	943 (78.2)	44 (58.7)	
Asian	139 (11.5)	21 (28)	
Others	124 (10.3)	10 (13.3)	
Sex			0.154
Female	574 (47.2)	29 (38.7)	
Male	641 (52.8)	46 (61.3)	
Birth mode			0.723
Vaginally born without IAP	636 (53.6)	35 (49.3)	
Vaginally born with IAP	273 (23)	16 (22.5)	
Scheduled C-section	113 (9.5)	7 (9.9)	
Emergency C-section	165 (13.9)	13 (18.3)	
Breastfeeding status (0-3 mo)			0.803
Formula fed	191 (15.7)	11 (14.7)	
Partially breast fed	314 (25.8)	17 (22.7)	
Exclusively breast fed	710 (58.4)	47 (62.7)	
Direct antibiotic exposure (0-12 mo)			0.878
No	841 (79.9)	56 (81.2)	
Yes	212 (20.1)	13 (18.8)	
Siblings			0.091

No	573 (48.3)	43 (58.9)	
Yes	613 (51.7)	30 (41.1)	
Solid food introduction (0-3 mo)			0.230
No	1108 (92.9)	72 (97.3)	
Yes	85 (7.1)	2 (2.7)	
Household pet exposure			0.048
No	587 (48.4)	48 (64)	
Only Prenatal	85 (7)	4 (5.3)	
Both Pre- and Postnatal	530 (43.7)	22 (29.3)	
Only Postnatal	12 (1)	1 (1.3)	
Trajectory			0.007
C1-C1	178 (14.7)	23 (30.7)	
C1-C2	461 (37.9)	22 (29.3)	
C2-C1	20 (1.6)	1 (1.3)	
C2-C2	556 (45.8)	29 (38.7)	
<i>C. difficile</i> colonization (early to late infancy)			0.303
A-P	309 (28.1)	18 (28.1)	
P-P	158 (14.4)	11 (17.2)	
P-A	166 (15.1)	14 (21.9)	
A-A	465 (42.3)	21 (32.8)	

Continuous data were shown in median (1st quartile, 3rd quartile), and differences were evaluated using Wilcoxon rank-sum tests. Categorical data were shown in n (% proportion), and differences were evaluated using Fisher's exact tests.

Table S7. Population characteristics stratified by inhalant sensitization at age 1 year.

	Inhalant sensitization at age 1 year		<i>p</i> value
	No	Yes	
Gestation age (week)	39 (38, 40)	39 (39, 40)	0.808
Location			<0.001
Vancouver	443 (32.4)	28 (60.9)	
Edmonton	296 (21.7)	9 (19.6)	
Winnipeg	628 (45.9)	9 (19.6)	
Maternal prepregnancy weight status			0.627
Normal	806 (61.3)	27 (65.9)	
Overweight or obese	509 (38.7)	14 (34.1)	
Maternal prenatal diabetes			0.721
No	1292 (95.2)	45 (97.8)	
Yes	65 (4.8)	1 (2.2)	
Maternal prenatal atopy			0.535
No	212 (15.9)	5 (10.9)	
Yes	1122 (84.1)	41 (89.1)	
Maternal prenatal food allergy			0.496
No	1006 (74.1)	32 (69.6)	
Yes	351 (25.9)	14 (30.4)	
Maternal prenatal asthma			0.089
No	1258 (92.2)	39 (84.8)	
Yes	106 (7.8)	7 (15.2)	
Maternal ethnicity			0.008
Caucasian	1058 (78.1)	27 (60)	
Asian	158 (11.7)	12 (26.7)	
Others	138 (10.2)	6 (13.3)	
Sex			0.368
Female	640 (46.8)	18 (39.1)	
Male	727 (53.2)	28 (60.9)	
Birth mode			0.356
Vaginally born without IAP	717 (53.8)	20 (45.5)	
Vaginally born with IAP	300 (22.5)	9 (20.5)	
Scheduled C-section	126 (9.5)	7 (15.9)	
Emergency C-section	190 (14.3)	8 (18.2)	
Breastfeeding status (0-3 mo)			0.618
Formula fed	218 (15.9)	5 (10.9)	
Partially breast fed	357 (26.1)	11 (23.9)	
Exclusively breast fed	792 (57.9)	30 (65.2)	
Direct antibiotic exposure (0-12 mo)			0.427
No	944 (79.9)	30 (75)	
Yes	237 (20.1)	10 (25)	
Siblings			0.541

No	651 (48.8)	19 (43.2)	
Yes	683 (51.2)	25 (56.8)	
Solid food introduction (0-3 mo)			0.358
No	1251 (93.1)	42 (97.7)	
Yes	93 (6.9)	1 (2.3)	
Household pet exposure			0.195
No	678 (49.6)	22 (47.8)	
Only Prenatal	95 (7)	0 (0)	
Both Pre- and Postnatal	577 (42.2)	24 (52.2)	
Only Postnatal	16 (1.2)	0 (0)	
Trajectory			0.861
C1-C1	210 (15.4)	7 (15.2)	
C1-C2	510 (37.3)	16 (34.8)	
C2-C1	21 (1.5)	1 (2.2)	
C2-C2	626 (45.8)	22 (47.8)	
<i>C. difficile</i> colonization (early to late infancy)			0.304
A-P	340 (27.6)	17 (41.5)	
P-P	179 (14.5)	4 (9.8)	
P-A	196 (15.9)	6 (14.6)	
A-A	518 (42)	14 (34.1)	

Continuous data were shown in median (1st quartile, 3rd quartile), and differences were evaluated using Wilcoxon rank-sum tests. Categorical data were shown in n (% proportion), and differences were evaluated using Fisher's exact tests.

Table S8. Population characteristics stratified by inhalant sensitization at age 3 years.

	Inhalant sensitization at age 3 years		<i>p</i> value
	No	Yes	
Gestation age (week)	39 (38, 40)	39 (38, 40)	0.119
Location			<0.001
Vancouver	364 (31.4)	64 (48.9)	
Edmonton	230 (19.8)	35 (26.7)	
Winnipeg	567 (48.8)	32 (24.4)	
Maternal prepregnancy weight status			0.066
Normal	687 (61.6)	89 (70.1)	
Overweight or obese	428 (38.4)	38 (29.9)	
Maternal prenatal diabetes			0.074
No	1108 (95.8)	120 (92.3)	
Yes	48 (4.2)	10 (7.7)	
Maternal prenatal atopy			0.796
No	177 (15.6)	18 (14.1)	
Yes	960 (84.4)	110 (85.9)	
Maternal prenatal food allergy			0.402
No	857 (74.1)	92 (70.8)	
Yes	299 (25.9)	38 (29.2)	
Maternal prenatal asthma			1.000
No	1065 (91.9)	120 (92.3)	
Yes	94 (8.1)	10 (7.7)	
Maternal ethnicity			<0.001
Caucasian	912 (79.1)	77 (59.2)	
Asian	121 (10.5)	39 (30)	
Others	120 (10.4)	14 (10.8)	
Sex			<0.001
Female	564 (48.6)	39 (29.8)	
Male	597 (51.4)	92 (70.2)	
Birth mode			0.070
Vaginally born without IAP	605 (53.4)	67 (52.8)	
Vaginally born with IAP	269 (23.7)	20 (15.7)	
Scheduled C-section	105 (9.3)	16 (12.6)	
Emergency C-section	154 (13.6)	24 (18.9)	
Breastfeeding status (0-3 mo)			0.723
Formula fed	180 (15.5)	22 (16.8)	
Partially breast fed	295 (25.4)	36 (27.5)	
Exclusively breast fed	686 (59.1)	73 (55.7)	
Direct antibiotic exposure (0-12 mo)			0.322
No	811 (80.3)	86 (76.1)	
Yes	199 (19.7)	27 (23.9)	
Siblings			0.578

No	557 (49.2)	60 (46.5)	
Yes	574 (50.8)	69 (53.5)	
Solid food introduction (0-3 mo)			0.585
No	1059 (93)	123 (94.6)	
Yes	80 (7)	7 (5.4)	
Household pet exposure			0.083
No	562 (48.4)	74 (56.5)	
Only Prenatal	83 (7.2)	6 (4.6)	
Both Pre- and Postnatal	505 (43.5)	48 (36.6)	
Only Postnatal	10 (0.9)	3 (2.3)	
Trajectory			0.152
C1-C1	173 (14.9)	28 (21.4)	
C1-C2	444 (38.2)	40 (30.5)	
C2-C1	19 (1.6)	2 (1.5)	
C2-C2	525 (45.2)	61 (46.6)	
<i>C. difficile</i> colonization (early to late infancy)			0.752
A-P	289 (27.7)	38 (31.4)	
P-P	150 (14.4)	19 (15.7)	
P-A	162 (15.5)	18 (14.9)	
A-A	441 (42.3)	46 (38)	

Continuous data were shown in median (1st quartile, 3rd quartile), and differences were evaluated using Wilcoxon rank-sum tests. Categorical data were shown in n (% proportion), and differences were evaluated using Fisher's exact tests.

Table S9. Population characteristics stratified by peanut sensitization at age 3 years.

	Peanut sensitization at age 3 years		<i>p</i> value
	No	Yes	
Gestation age (week)	39 (38, 40)	39 (38, 40)	0.259
Location			0.381
Edmonton	252 (20.3)	13 (28.9)	
Vancouver	409 (33)	13 (28.9)	
Winnipeg	578 (46.7)	19 (42.2)	
Maternal prepregnancy weight status			0.625
Normal	747 (62.6)	24 (58.5)	
Overweight or obese	447 (37.4)	17 (41.5)	
Maternal prenatal diabetes			0.718
No	1178 (95.5)	44 (97.8)	
Yes	55 (4.5)	1 (2.2)	
Maternal prenatal atopy			0.67
No	186 (15.3)	8 (18.2)	
Yes	1027 (84.7)	36 (81.8)	
Maternal prenatal food allergy			1
No	912 (74)	33 (73.3)	
Yes	321 (26)	12 (26.7)	
Maternal prenatal asthma			0.576
No	1134 (91.7)	43 (95.6)	
Yes	102 (8.3)	2 (4.4)	
Maternal ethnicity			<0.001
Caucasian	958 (77.9)	24 (53.3)	
Asian	144 (11.7)	15 (33.3)	
Others	128 (10.4)	6 (13.3)	
Sex			0.548
Female	581 (46.9)	19 (42.2)	
Male	658 (53.1)	26 (57.8)	
Birth mode			0.43
Vaginal, IAP-	646 (53.3)	23 (54.8)	
Vaginal, IAP+	282 (23.3)	6 (14.3)	
Cesarean, scheduled	113 (9.3)	5 (11.9)	
Cesarean, emergency	170 (14)	8 (19)	
Breastfeeding status (0-3 mo)			0.932
Formula fed	196 (15.8)	6 (13.3)	
Partially breast fed	316 (25.5)	11 (24.4)	
Exclusively breast fed	727 (58.7)	28 (62.2)	
Direct antibiotic exposure (0-12 mo)			0.547
No	860 (79.9)	34 (85)	
Yes	217 (20.1)	6 (15)	
Siblings			0.064

No	585 (48.4)	28 (63.6)	
Yes	624 (51.6)	16 (36.4)	
Solid food introduction (0-3 mo)			0.764
No	1132 (93)	42 (95.5)	
Yes	85 (7)	2 (4.5)	
Household pet exposure			0.233
No	605 (48.9)	27 (60)	
Only Prenatal	85 (6.9)	3 (6.7)	
Both Pre- and Postnatal	536 (43.3)	14 (31.1)	
Only Postnatal	12 (1)	1 (2.2)	
Trajectory			0.009
C1-C1	184 (14.9)	16 (35.6)	
C1-C2	468 (37.8)	13 (28.9)	
C2-C1	21 (1.7)	0 (0)	
C2-C2	566 (45.7)	16 (35.6)	
<i>C. difficile</i> colonization (early to late infancy)			0.242
A-P	312 (27.9)	13 (35.1)	
P-P	161 (14.4)	6 (16.2)	
P-A	172 (15.4)	8 (21.6)	
A-A	474 (42.4)	10 (27)	

Continuous data were shown in median (1st quartile, 3rd quartile), and differences were evaluated using Wilcoxon rank-sum tests. Categorical data were shown in n (% proportion), and differences were evaluated using Fisher's exact tests.

Table S10. Median (interquartile range) values of alpha diversity indices in different groups.

Alpha diversity indice	Taxonomic group	Early-infancy		Late-infancy	
		Cluster 1	Cluster 2	Cluster 1	Cluster 2
Chao1	Overall	173.1 (143.3, 205.3) ^a	204.9 (173.1, 243.2) ^b	293.6 (253.3, 335.3) ^c	343.9 (293.9, 391) ^d
	Actinobacteria	18 (11, 22) ^a	17 (11.3, 21.6) ^{ab}	19.5 (14.1, 24) ^c	16 (11.5, 20.5) ^b
	Bacteroidetes	10 (5.3, 22) ^a	75.7 (55.2, 99.5) ^b	19.5 (9.6, 32.3) ^c	96.1 (68.1, 122.7) ^d
	Firmicutes	73.4 (54.1, 98.5) ^a	60.2 (41, 81.8) ^b	184.2 (148.7, 229.6) ^c	193.2 (148.3, 237.5) ^c
	Proteobacteria	59.5 (47.2, 74.4) ^a	46 (32.9, 61.2) ^b	53.1 (39.5, 68.2) ^c	30.8 (17.5, 48) ^d
Observed OTUs	Overall	129 (109, 149) ^a	148 (128.5, 174) ^b	204 (178, 231.8) ^c	240 (208, 276) ^d
	Actinobacteria	16 (9, 20) ^a	14 (9, 18) ^b	16 (12, 20) ^c	13 (10, 17) ^d
	Bacteroidetes	6 (4, 12) ^a	58 (44, 78) ^b	10 (7, 18.8) ^c	75 (53, 95) ^d
	Firmicutes	53 (40, 68.2) ^a	39 (27, 54) ^b	132.5 (107.2, 159.8) ^c	130 (98, 164) ^c
	Proteobacteria	49 (37, 60) ^a	32 (23, 41) ^b	34 (26, 45.8) ^c	18 (12, 26) ^d
PD whole tree	Overall	9.2 (8.2, 10.6) ^a	9.5 (8.4, 10.7) ^b	14.6 (13.1, 16.1) ^c	15.7 (13.9, 18) ^d
	Actinobacteria	1.7 (1.3, 1.9) ^a	1.6 (1.4, 1.8) ^a	1.7 (1.5, 1.9) ^b	1.6 (1.5, 1.8) ^a
	Bacteroidetes	1.6 (1.4, 1.9) ^a	2.9 (2.5, 3.4) ^b	1.9 (1.7, 2.3) ^c	3.5 (3, 4.1) ^d
	Firmicutes	4.2 (3.5, 5.1) ^a	3.7 (3.1, 4.5) ^b	8.5 (7.3, 10.1) ^c	9 (7.3, 10.9) ^c
	Proteobacteria	2.4 (2, 2.8) ^a	2 (1.7, 2.3) ^b	2.3 (1.9, 2.7) ^c	1.9 (1.6, 2.2) ^d
Shannon	Overall	3.1 (2.6, 3.5) ^a	3.1 (2.6, 3.6) ^b	4.2 (3.9, 4.6) ^c	4.3 (3.8, 4.7) ^c
	Actinobacteria	1.4 (1.1, 1.8) ^a	1.5 (1.1, 1.8) ^a	1.6 (1.3, 1.9) ^b	1.7 (1.4, 2) ^c
	Bacteroidetes	1.9 (1.2, 2.3) ^a	1.6 (1, 2.3) ^b	2 (1, 2.7) ^c	2.4 (1.7, 2.9) ^d
	Firmicutes	2.5 (2, 3) ^a	2.5 (1.9, 3.1) ^a	3.9 (3.5, 4.3) ^b	4.3 (3.7, 4.7) ^c
	Proteobacteria	1.4 (1.1, 1.8) ^a	1.5 (1.2, 1.9) ^b	1.9 (1.5, 2.2) ^c	1.8 (1.4, 2.2) ^d
Simpson	Overall	0.8 (0.7, 0.8) ^a	0.8 (0.7, 0.8) ^a	0.9 (0.9, 0.9) ^b	0.9 (0.8, 0.9) ^c
	Actinobacteria	0.4 (0.3, 0.6) ^a	0.4 (0.3, 0.6) ^a	0.5 (0.4, 0.6) ^b	0.5 (0.4, 0.6) ^c
	Bacteroidetes	0.7 (0.5, 0.8) ^a	0.5 (0.2, 0.7) ^b	0.6 (0.3, 0.8) ^a	0.7 (0.5, 0.8) ^c
	Firmicutes	0.7 (0.6, 0.8) ^a	0.7 (0.6, 0.8) ^a	0.9 (0.8, 0.9) ^b	0.9 (0.8, 0.9) ^c
	Proteobacteria	0.3 (0.3, 0.5) ^a	0.4 (0.3, 0.6) ^b	0.6 (0.4, 0.7) ^c	0.6 (0.4, 0.7) ^c

^{a-d} Means within a row with different superscripts differ (Benjamini-Hochberg adjusted $P < 0.1$).

Table S11. Multiple logistic regression for the association between atopic sensitization at age 1 year and microbiota development trajectories.

	Model 1 (BIC: 1052.9)		Model 2 (BIC: 934.2)		Model 3 (BIC: 891.4)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
A. Developmental trajectory of microbiota (Ref.: C2-C2)						
C1-C2	1.14 (0.79, 1.64)	0.492	1.17 (0.75, 1.82)	0.484	1.27 (0.80, 2.00)	0.312
C1-C1	1.96 (1.28, 3.00)	0.002	1.93 (1.17, 3.19)	0.010	1.63 (0.96, 2.78)	0.071
C2-C1	0.41 (0.06, 3.12)	0.391	0.00 (0.00, N.A.)	0.998	0.00 (0.00, N.A.)	0.998
B. Developmental trajectory of microbiota (Ref.: C1-C2)						
C2-C2	0.88 (0.61, 1.27)	0.492	0.85 (0.55, 1.33)	0.484	0.79 (0.50, 1.25)	0.312
C1-C1	1.72 (1.11, 2.66)	0.014	1.65 (1.01, 2.70)	0.046	1.29 (0.77, 2.17)	0.338
C2-C1	0.36 (0.05, 2.75)	0.326	0.00 (0.00, N.A.)	0.998	0.00 (0.00, N.A.)	0.998
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			0.87 (0.50, 1.52)	0.631	0.89 (0.50, 1.57)	0.682
Cesarean, scheduled			1.14 (0.59, 2.17)	0.701	1.20 (0.61, 2.33)	0.597
Vaginal, IAP+			0.78 (0.48, 1.25)	0.303	0.81 (0.49, 1.34)	0.412
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.84 (0.49, 1.45)	0.537	1.02 (0.58, 1.78)	0.941
Partial			0.94 (0.61, 1.47)	0.801	0.94 (0.60, 1.49)	0.809
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			0.81 (0.50, 1.32)	0.398	0.88 (0.54, 1.44)	0.611
Siblings (Ref.: Yes)						
No			1.08 (0.73, 1.58)	0.700	1.05 (0.70, 1.57)	0.804
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			1.69 (1.15, 2.48)	0.008	1.37 (0.91, 2.07)	0.136
Only prenatal			0.86 (0.35, 2.12)	0.749	0.90 (0.36, 2.28)	0.831
Only postnatal			0.98 (0.12, 7.93)	0.985	0.84 (0.10, 6.94)	0.871
1st sampling time (mo)			1.02 (0.83, 1.24)	0.870	1.23 (0.99, 1.52)	0.060
2nd sampling time (mo)			0.77 (0.63, 0.95)	0.013	0.75 (0.61, 0.93)	0.009
Maternal race/ethnicity (Ref.: White)						
Others					1.11 (0.58, 2.14)	0.757
Asian					3.25 (2.04, 5.18)	<0.001
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.10 (0.01, 0.74)	0.024
Maternal prenatal food allergy (Ref.: No)						
Yes					1.56 (1.04, 2.35)	0.031

* Models A and B were run using different reference categories. Covariate adjustment was identical for models 2A and 2B, and for 3A and 3B.

Table S12. Multiple logistic regression for the association between and atopic sensitization at age 3 years and microbiota developmental trajectories.

	Model 1 (BIC: 1010.2)		Model 2 (BIC: 908.4)		Model 3 (BIC: 895.5)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
A. Developmental trajectory of microbiota (Ref.: C2-C2)						
C1-C2	0.83 (0.57, 1.21)	0.329	0.80 (0.50, 1.26)	0.333	0.85 (0.53, 1.35)	0.484
C1-C1	1.69 (1.10, 2.59)	0.016	1.90 (1.15, 3.14)	0.013	1.57 (0.93, 2.65)	0.094
C2-C1	1.17 (0.34, 4.07)	0.804	0.90 (0.20, 4.13)	0.888	0.76 (0.16, 3.66)	0.734
B. Developmental trajectory of microbiota (Ref.: C1-C2)						
C2-C2	1.21 (0.83, 1.77)	0.329	1.25 (0.79, 1.98)	0.333	1.18 (0.74, 1.89)	0.484
C1-C1	2.04 (1.30, 3.22)	0.002	2.38 (1.43, 3.96)	0.001	1.85 (1.09, 3.15)	0.022
C2-C1	1.42 (0.40, 4.97)	0.588	1.12 (0.24, 5.23)	0.881	0.90 (0.19, 4.36)	0.896
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			1.10 (0.64, 1.91)	0.725	1.01 (0.57, 1.77)	0.973
Cesarean, scheduled			1.47 (0.78, 2.76)	0.234	1.36 (0.70, 2.62)	0.363
Vaginal, IAP+			0.68 (0.41, 1.13)	0.136	0.65 (0.39, 1.09)	0.106
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			1.13 (0.67, 1.90)	0.640	1.14 (0.66, 1.94)	0.642
Partial			1.10 (0.71, 1.72)	0.662	1.03 (0.65, 1.62)	0.909
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			1.30 (0.84, 2.03)	0.237	1.35 (0.86, 2.12)	0.194
Siblings (Ref.: Yes)						
No			1.15 (0.78, 1.69)	0.477	1.21 (0.81, 1.80)	0.345
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			1.63 (1.11, 2.41)	0.014	1.36 (0.90, 2.05)	0.144
Only prenatal			0.66 (0.25, 1.73)	0.392	0.63 (0.24, 1.70)	0.364
Only postnatal			2.81 (0.56, 14.23)	0.211	2.41 (0.45, 12.89)	0.303
1st sampling time (mo)			1.07 (0.88, 1.30)	0.497	1.19 (0.96, 1.47)	0.119
2nd sampling time (mo)			1.01 (0.85, 1.20)	0.937	1.01 (0.85, 1.20)	0.909
Maternal race/ethnicity (Ref.: White)						
Others					1.88 (1.07, 3.30)	0.028
Asian					3.25 (2.04, 5.19)	<0.001
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.67 (0.28, 1.62)	0.372
Maternal prenatal food allergy (Ref.: No)						
Yes					1.19 (0.78, 1.80)	0.423

* Models A and B were run using different reference categories. Covariate adjustment was identical for models 2A and 2B, and for 3A and 3B.

Table S13. Multiple logistic regression for the association between food sensitization at age 1 year and microbiota developmental trajectories.

	Model 1 (BIC: 901.0)		Model 2 (BIC: 828.4)		Model 3 (BIC: 785.7)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
A. Developmental trajectory of microbiota (Ref.: C2-C2)						
C1-C2	1.10 (0.73, 1.66)	0.644	1.22 (0.76, 1.98)	0.413	1.36 (0.82, 2.24)	0.236
C1-C1	2.09 (1.31, 3.31)	0.002	2.05 (1.19, 3.53)	0.010	1.77 (0.99, 3.16)	0.052
C2-C1	0.00 (0.00, N.A.)	0.998	0.00 (0.00, N.A.)	0.998	0.00 (0.00, N.A.)	0.998
B. Developmental trajectory of microbiota (Ref.: C1-C2)						
C2-C2	0.91 (0.60, 1.37)	0.644	0.82 (0.51, 1.32)	0.413	0.74 (0.45, 1.22)	0.236
C1-C1	1.89 (1.18, 3.04)	0.008	1.67 (0.98, 2.85)	0.058	1.31 (0.74, 2.30)	0.349
C2-C1	0.00 (0.00, N.A.)	0.998	0.00 (0.00, N.A.)	0.998	0.00 (0.00, N.A.)	0.998
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			0.68 (0.37, 1.27)	0.227	0.69 (0.36, 1.30)	0.251
Cesarean, scheduled			0.91 (0.44, 1.88)	0.801	0.96 (0.46, 2.02)	0.916
Vaginal, IAP+			0.73 (0.43, 1.22)	0.230	0.75 (0.44, 1.31)	0.314
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			1.02 (0.58, 1.82)	0.935	1.30 (0.72, 2.35)	0.391
Partial			1.13 (0.70, 1.81)	0.613	1.15 (0.70, 1.88)	0.579
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			0.85 (0.50, 1.44)	0.534	0.93 (0.54, 1.60)	0.803
Siblings (Ref.: Yes)						
No			1.22 (0.81, 1.86)	0.344	1.21 (0.78, 1.87)	0.399
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			1.86 (1.21, 2.86)	0.005	1.51 (0.95, 2.40)	0.078
Only prenatal			1.16 (0.47, 2.91)	0.746	1.23 (0.48, 3.17)	0.664
Only postnatal			1.22 (0.15, 9.96)	0.851	1.12 (0.13, 9.35)	0.916
1st sampling time (mo)			0.97 (0.77, 1.22)	0.782	1.19 (0.93, 1.52)	0.158
2nd sampling time (mo)			0.79 (0.63, 0.98)	0.034	0.76 (0.60, 0.96)	0.021
Maternal race/ethnicity (Ref.: White)						
Others					1.15 (0.56, 2.35)	0.695
Asian					3.12 (1.89, 5.15)	<0.001
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.00 (0.00, N.A.)	0.997
Maternal prenatal food allergy (Ref.: No)						
Yes					1.64 (1.06, 2.55)	0.027

* * Models A and B were run using different reference categories. Covariate adjustment was identical for models 2A and 2B, and for 3A and 3B.

Table S14. Multiple logistic regression for the association between food sensitization at age 3 years and microbiota developmental trajectories.

	Model 1 (BIC: 589.4)		Model 2 (BIC: 577.4)		Model 3 (BIC: 582.4)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
A. Developmental trajectory of microbiota (Ref.: C2-C2)						
C1-C2	0.92 (0.52, 1.61)	0.759	0.90 (0.46, 1.75)	0.753	0.92 (0.47, 1.81)	0.816
C1-C1	2.48 (1.40, 4.39)	0.002	2.34 (1.20, 4.56)	0.013	1.86 (0.93, 3.72)	0.079
C2-C1	0.96 (0.12, 7.39)	0.968	1.04 (0.13, 8.46)	0.968	1.01 (0.12, 8.50)	0.992
B. Developmental trajectory of microbiota (Ref.: C1-C2)						
C2-C2	1.09 (0.62, 1.93)	0.759	1.11 (0.57, 2.17)	0.753	1.08 (0.55, 2.13)	0.816
C1-C1	2.71 (1.47, 4.98)	0.001	2.60 (1.33, 5.09)	0.005	2.02 (1.00, 4.05)	0.049
C2-C1	1.05 (0.13, 8.17)	0.965	1.16 (0.14, 9.49)	0.888	1.09 (0.13, 9.28)	0.933
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			1.01 (0.48, 2.15)	0.969	0.94 (0.44, 2.03)	0.880
Cesarean, scheduled			1.30 (0.52, 3.25)	0.581	1.36 (0.54, 3.44)	0.519
Vaginal, IAP+			0.79 (0.40, 1.55)	0.486	0.80 (0.40, 1.59)	0.523
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.84 (0.39, 1.81)	0.655	0.88 (0.40, 1.91)	0.743
Partial			0.89 (0.47, 1.69)	0.726	0.82 (0.43, 1.58)	0.559
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			1.09 (0.57, 2.07)	0.801	1.07 (0.55, 2.06)	0.846
Siblings (Ref.: Yes)						
No			1.52 (0.88, 2.62)	0.130	1.71 (0.98, 2.98)	0.060
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			2.11 (1.21, 3.68)	0.009	1.88 (1.04, 3.38)	0.036
Only prenatal			0.67 (0.15, 2.98)	0.596	0.70 (0.15, 3.18)	0.643
Only postnatal			0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.999
1st sampling time (mo)			0.96 (0.70, 1.32)	0.817	1.08 (0.78, 1.50)	0.646
2nd sampling time (mo)			0.77 (0.57, 1.04)	0.092	0.80 (0.59, 1.07)	0.135
Maternal race/ethnicity (Ref.: White)						
Others					2.11 (0.99, 4.50)	0.053
Asian					2.66 (1.42, 4.96)	0.002
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.25 (0.03, 1.99)	0.191
Maternal prenatal food allergy (Ref.: No)						
Yes					0.76 (0.41, 1.42)	0.391

* Models A and B were run using different reference categories. Covariate adjustment was identical for models 2A and 2B, and for 3A and 3B.

Table S15. Multiple logistic regression for the association between inhalant sensitization at age 1 year and microbiota developmental trajectories.

	Model 1 (BIC: 434.4)		Model 2 (BIC: 420.8)		Model 3 (BIC: 423.6)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
A. Developmental trajectory of microbiota (Ref.: C2-C2)						
C1-C2	0.89 (0.46, 1.72)	0.734	0.61 (0.26, 1.41)	0.245	0.72 (0.30, 1.69)	0.447
C1-C1	0.95 (0.40, 2.25)	0.905	0.80 (0.30, 2.15)	0.660	0.71 (0.25, 2.00)	0.512
C2-C1	1.35 (0.17, 10.53)	0.772	0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.998
B. Developmental trajectory of microbiota (Ref.: C1-C2)						
C2-C2	1.12 (0.58, 2.15)	0.734	1.64 (0.71, 3.79)	0.245	1.40 (0.59, 3.30)	0.447
C1-C1	1.06 (0.43, 2.62)	0.895	1.31 (0.48, 3.59)	0.593	0.98 (0.35, 2.81)	0.977
C2-C1	1.52 (0.19, 11.99)	0.692	0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.998
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			2.63 (0.99, 6.98)	0.052	2.82 (1.02, 7.79)	0.046
Cesarean, scheduled			2.64 (0.83, 8.39)	0.100	2.78 (0.85, 9.07)	0.090
Vaginal, IAP+			1.28 (0.53, 3.09)	0.578	1.46 (0.59, 3.61)	0.417
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.34 (0.10, 1.15)	0.083	0.38 (0.11, 1.30)	0.123
Partial			0.49 (0.20, 1.19)	0.114	0.50 (0.20, 1.25)	0.138
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			1.11 (0.49, 2.50)	0.808	1.24 (0.54, 2.83)	0.615
Siblings (Ref.: Yes)						
No			0.66 (0.32, 1.36)	0.259	0.61 (0.29, 1.30)	0.206
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			0.75 (0.38, 1.48)	0.409	0.60 (0.29, 1.26)	0.177
Only prenatal			0.00 (0.00, N.A.)	0.997	0.00 (0.00, N.A.)	0.997
Only postnatal			0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.999
1st sampling time (mo)			1.07 (0.75, 1.53)	0.708	1.20 (0.81, 1.76)	0.368
2nd sampling time (mo)			0.75 (0.51, 1.11)	0.146	0.66 (0.42, 1.03)	0.069
Maternal race/ethnicity (Ref.: White)						
Others					1.92 (0.69, 5.35)	0.215
Asian					3.10 (1.28, 7.51)	0.012
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.51 (0.06, 4.34)	0.538
Maternal prenatal food allergy (Ref.: No)						
Yes					1.46 (0.69, 3.09)	0.322

* Models A and B were run using different reference categories. Covariate adjustment was identical for models 2A and 2B, and for 3A and 3B.

Table S16. Multiple logistic regression for the association between inhalant sensitization at age 3 years and microbiota developmental trajectories.

	Model 1 (BIC: 871.6)		Model 2 (BIC: 781.6)		Model 3 (BIC: 778.9)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
A. Developmental trajectory of microbiota (Ref.: C2-C2)						
C1-C2	0.78 (0.51, 1.18)	0.233	0.62 (0.37, 1.04)	0.073	0.67 (0.39, 1.14)	0.136
C1-C1	1.39 (0.86, 2.25)	0.175	1.45 (0.81, 2.57)	0.207	1.24 (0.68, 2.24)	0.480
C2-C1	0.91 (0.21, 3.98)	0.896	0.54 (0.07, 4.29)	0.563	0.47 (0.06, 3.83)	0.478
B. Developmental trajectory of microbiota (Ref.: C1-C2)						
C2-C2	1.29 (0.85, 1.96)	0.233	1.61 (0.96, 2.71)	0.073	1.50 (0.88, 2.54)	0.136
C1-C1	1.80 (1.07, 3.00)	0.025	2.33 (1.29, 4.20)	0.005	1.85 (1.01, 3.40)	0.047
C2-C1	1.17 (0.26, 5.20)	0.838	0.88 (0.11, 7.01)	0.900	0.70 (0.08, 5.80)	0.740
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			1.64 (0.90, 2.98)	0.104	1.50 (0.81, 2.76)	0.197
Cesarean, scheduled			1.98 (1.00, 3.92)	0.050	1.77 (0.87, 3.60)	0.117
Vaginal, IAP+			0.77 (0.43, 1.38)	0.379	0.73 (0.41, 1.32)	0.297
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			1.31 (0.75, 2.29)	0.349	1.33 (0.74, 2.37)	0.335
Partial			1.14 (0.70, 1.87)	0.597	1.10 (0.67, 1.83)	0.701
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			1.43 (0.89, 2.32)	0.142	1.48 (0.91, 2.42)	0.115
Siblings (Ref.: Yes)						
No			0.89 (0.57, 1.37)	0.586	0.89 (0.57, 1.39)	0.605
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			1.24 (0.81, 1.91)	0.320	0.98 (0.62, 1.55)	0.938
Only prenatal			0.59 (0.20, 1.74)	0.342	0.56 (0.19, 1.66)	0.298
Only postnatal			3.62 (0.70, 18.66)	0.125	2.94 (0.54, 15.90)	0.210
1st sampling time (mo)			1.14 (0.92, 1.40)	0.238	1.25 (1.00, 1.57)	0.055
2nd sampling time (mo)			1.04 (0.86, 1.25)	0.707	1.04 (0.86, 1.25)	0.695
Maternal race/ethnicity (Ref.: White)						
Others					1.51 (0.78, 2.91)	0.221
Asian					3.39 (2.02, 5.69)	<0.001
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.67 (0.26, 1.74)	0.410
Maternal prenatal food allergy (Ref.: No)						
Yes					1.29 (0.81, 2.05)	0.276

* Models A and B were run using different reference categories. Covariate adjustment was identical for models 2A and 2B, and for 3A and 3B.

Table S17. Multiple logistic regression for the association between peanut sensitization at age 3 years and microbiota developmental trajectories.

	Model 1 (BIC: 406.2)		Model 2 (BIC: 410.3)		Model 3 (BIC: 416.9)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
A. Developmental trajectory of microbiota (Ref.: C2-C2)						
C1-C2	0.98 (0.47, 2.06)	0.963	1.33 (0.55, 3.25)	0.529	1.40 (0.57, 3.46)	0.465
C1-C1	3.08 (1.51, 6.27)	0.002	3.80 (1.58, 9.09)	0.003	2.82 (1.13, 7.01)	0.026
C2-C1	0.00 (0.00, N.A.)	0.998	0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.998
B. Developmental trajectory of microbiota (Ref.: C1-C2)						
C2-C2	1.02 (0.48, 2.14)	0.963	0.75 (0.31, 1.83)	0.529	0.71 (0.29, 1.77)	0.465
C1-C1	3.13 (1.48, 6.64)	0.003	2.85 (1.25, 6.51)	0.013	2.01 (0.85, 4.78)	0.114
C2-C1	0.00 (0.00, N.A.)	0.998	0.00 (0.00, N.A.)	0.998	0.00 (0.00, N.A.)	0.998
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			0.69 (0.27, 1.77)	0.439	0.62 (0.23, 1.66)	0.343
Cesarean, scheduled			1.41 (0.47, 4.22)	0.534	1.53 (0.50, 4.68)	0.453
Vaginal, IAP+			0.33 (0.11, 0.98)	0.046	0.33 (0.11, 0.99)	0.049
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.60 (0.20, 1.80)	0.361	0.65 (0.21, 1.98)	0.446
Partial			0.95 (0.42, 2.14)	0.895	0.82 (0.35, 1.92)	0.642
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			0.81 (0.33, 2.01)	0.650	0.84 (0.33, 2.13)	0.721
Siblings (Ref.: Yes)						
No			1.90 (0.93, 3.92)	0.080	2.24 (1.05, 4.78)	0.036
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			1.89 (0.92, 3.87)	0.084	1.55 (0.71, 3.35)	0.271
Only prenatal			0.45 (0.06, 3.63)	0.451	0.40 (0.05, 3.41)	0.403
Only postnatal			0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.999
1st sampling time (mo)			0.96 (0.64, 1.44)	0.840	1.08 (0.70, 1.68)	0.724
2nd sampling time (mo)			0.84 (0.58, 1.23)	0.366	0.85 (0.59, 1.23)	0.397
Maternal race/ethnicity (Ref.: White)						
Others					2.96 (1.10, 7.96)	0.032
Asian					3.97 (1.80, 8.78)	0.001
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.60 (0.07, 5.18)	0.646
Maternal prenatal food allergy (Ref.: No)						
Yes					1.14 (0.54, 2.43)	0.733

* Models A and B were run using different reference categories. Covariate adjustment was identical for models 2A and 2B, and for 3A and 3B.

Table S18. Multiple logistic regression for the association between atopic sensitization at age 1 years and early infancy microbiota clusters.

	Model 1 (BIC: 1045.2)		Model 2 (BIC: 947.9)		Model 3 (BIC: 901.4)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
Early infancy cluster membership (Ref.: C2)						
C1	1.39 (1.00, 1.93)	0.047	1.35 (0.91, 1.98)	0.133	1.33 (0.88, 1.99)	0.172
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			0.91 (0.53, 1.56)	0.721	0.93 (0.53, 1.62)	0.786
Cesarean, scheduled			1.15 (0.62, 2.14)	0.664	1.18 (0.62, 2.25)	0.608
Vaginal, IAP+			0.77 (0.48, 1.24)	0.281	0.79 (0.48, 1.29)	0.349
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.89 (0.53, 1.51)	0.670	1.10 (0.64, 1.89)	0.740
Partial			0.93 (0.61, 1.44)	0.756	0.94 (0.60, 1.48)	0.797
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			0.78 (0.48, 1.25)	0.301	0.84 (0.51, 1.37)	0.483
Siblings (Ref.: Yes)						
No			1.15 (0.79, 1.67)	0.464	1.10 (0.74, 1.62)	0.631
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			1.57 (1.07, 2.29)	0.020	1.28 (0.85, 1.92)	0.230
Only prenatal			0.91 (0.40, 2.11)	0.830	0.98 (0.41, 2.32)	0.965
Only postnatal			0.91 (0.11, 7.30)	0.926	0.77 (0.09, 6.40)	0.805
1st sampling time (mo)			0.95 (0.78, 1.15)	0.572	1.14 (0.93, 1.41)	0.204
Maternal race/ethnicity (Ref.: White)						
Others					1.13 (0.59, 2.16)	0.714
Asian					3.29 (2.10, 5.14)	<0.001
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.09 (0.01, 0.65)	0.017
Maternal prenatal food allergy (Ref.: No)						
Yes					1.54 (1.04, 2.29)	0.033

Table S19. Multiple logistic regression for the association between atopic sensitization at age 1 year and late infancy microbiota clusters.

	Model 1 (BIC: 1042.4)		Model 2 (BIC: 922.0)		Model 3 (BIC: 882.7)	
	Coefficient	95% CI	p	Coefficient	95% CI	p
Late infancy cluster membership (Ref.: C2)						
C1	1.69	(1.15, 2.48)	0.007	1.57	(1.01, 2.44)	0.045
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency				0.97	(0.57, 1.65)	0.914
Cesarean, scheduled				1.16	(0.63, 2.15)	0.628
Vaginal, IAP+				0.79	(0.49, 1.27)	0.323
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No				0.83	(0.48, 1.42)	0.496
Partial				0.90	(0.58, 1.40)	0.650
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes				0.81	(0.50, 1.32)	0.405
Siblings (Ref.: Yes)						
No				1.08	(0.74, 1.58)	0.682
Household pet exposure (Ref.: Both prenatal and postnatal)						
No				1.67	(1.14, 2.46)	0.009
Only prenatal				0.85	(0.35, 2.08)	0.718
Only postnatal				0.95	(0.12, 7.65)	0.960
2nd sampling time (mo)				0.77	(0.63, 0.94)	0.009
Maternal race/ethnicity (Ref.: White)						
Others					1.09	(0.57, 2.10)
Asian					3.05	(1.93, 4.83)
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.13	(0.02, 0.94)
Maternal prenatal food allergy (Ref.: No)						
Yes					1.51	(1.01, 2.25)

Table S20. Multiple logistic regression for the association between atopic sensitization at age 3 years and early infancy microbiota clusters.

	Model 1 (BIC: 1005.2)		Model 2 (BIC: 923.8)		Model 3 (BIC: 900.8)	
	Coefficient	95% CI	p	Coefficient	95% CI	p
Early infancy cluster membership (Ref.: C2)						
C1	1.06	(0.76, 1.47)	0.740	1.05	(0.71, 1.56)	0.804
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency				1.14	(0.66, 1.95)	0.640
Cesarean, scheduled				1.37	(0.75, 2.51)	0.309
Vaginal, IAP+				0.69	(0.42, 1.13)	0.143
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No				1.03	(0.62, 1.71)	0.912
Partial				1.03	(0.67, 1.58)	0.901
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes				1.24	(0.80, 1.92)	0.332
Siblings (Ref.: Yes)						
No				1.19	(0.82, 1.74)	0.354
Household pet exposure (Ref.: Both prenatal and postnatal)						
No				1.62	(1.11, 2.36)	0.013
Only prenatal				0.76	(0.31, 1.85)	0.548
Only postnatal				2.42	(0.48, 12.15)	0.283
1st sampling time (mo)				1.06	(0.88, 1.27)	0.553
Maternal race/ethnicity (Ref.: White)						
Others					1.92	(1.10, 3.34)
Asian					3.78	(2.41, 5.92)
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.62	(0.26, 1.50)
Maternal prenatal food allergy (Ref.: No)						
Yes					1.18	(0.78, 1.78)

Table S21. Multiple logistic regression for the association between atopic sensitization at age 3 years and late infancy microbiota clusters.

	Model 1 (BIC: 997.2)		Model 2 (BIC: 891.8)		Model 3 (BIC: 880.6)				
	Coefficient	95% CI	p	Coefficient	95% CI	p			
Late infancy cluster membership (Ref.: C2)									
C1	1.78	(1.21, 2.62)	0.003	1.98	(1.28, 3.07)	0.002	1.60	(1.01, 2.53)	0.043
Birth mode (Ref.: Vaginal, IAP-)									
Cesarean, emergency				1.07	(0.64, 1.81)	0.794	1.02	(0.60, 1.75)	0.936
Cesarean, scheduled				1.29	(0.71, 2.34)	0.397	1.19	(0.64, 2.21)	0.582
Vaginal, IAP+				0.66	(0.40, 1.09)	0.108	0.64	(0.38, 1.07)	0.088
Breastfeeding status (0-3 mo) (Ref.: Exclusive)									
No				1.14	(0.68, 1.91)	0.620	1.13	(0.66, 1.92)	0.665
Partial				1.11	(0.72, 1.71)	0.645	1.02	(0.65, 1.60)	0.931
Direct antibiotic exposure (0-12 mo) (Ref.: No)									
Yes				1.29	(0.83, 2.00)	0.257	1.32	(0.84, 2.07)	0.224
Siblings (Ref.: Yes)									
No				1.13	(0.77, 1.65)	0.545	1.16	(0.79, 1.73)	0.450
Household pet exposure (Ref.: Both prenatal and postnatal)									
No				1.60	(1.08, 2.35)	0.018	1.33	(0.89, 2.01)	0.166
Only prenatal				0.63	(0.24, 1.66)	0.351	0.59	(0.22, 1.58)	0.296
Only postnatal				2.72	(0.54, 13.70)	0.225	2.23	(0.42, 11.72)	0.345
2nd sampling time (mo)				1.02	(0.86, 1.20)	0.835	1.03	(0.87, 1.22)	0.708
Maternal race/ethnicity (Ref.: White)									
Others							1.89	(1.08, 3.30)	0.027
Asian							3.17	(1.99, 5.03)	<0.001
Solid food introduction (0-3 mo) (Ref.: No)									
Yes							0.81	(0.35, 1.87)	0.619
Maternal prenatal food allergy (Ref.: No)									
Yes							1.16	(0.77, 1.76)	0.480

Table S22. Multiple logistic regression for the association between food sensitization at age 1 year and early infancy microbiota clusters.

	Model 1 (BIC: 897.0)		Model 2 (BIC: 833.4)		Model 3 (BIC: 790.9)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
Early infancy cluster membership (Ref.: C2)						
C1	1.42 (0.99, 2.05)	0.056	1.44 (0.94, 2.20)	0.091	1.45 (0.93, 2.27)	0.101
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			0.70 (0.38, 1.29)	0.254	0.72 (0.38, 1.34)	0.296
Cesarean, scheduled			0.85 (0.41, 1.73)	0.647	0.87 (0.42, 1.81)	0.706
Vaginal, IAP+			0.71 (0.42, 1.19)	0.198	0.73 (0.42, 1.25)	0.256
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			1.02 (0.58, 1.80)	0.948	1.30 (0.72, 2.34)	0.378
Partial			1.12 (0.71, 1.78)	0.629	1.15 (0.71, 1.86)	0.561
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			0.82 (0.49, 1.39)	0.468	0.90 (0.53, 1.54)	0.706
Siblings (Ref.: Yes)						
No			1.31 (0.87, 1.97)	0.191	1.27 (0.83, 1.95)	0.269
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			1.76 (1.16, 2.69)	0.009	1.46 (0.93, 2.30)	0.102
Only prenatal			1.25 (0.53, 2.94)	0.604	1.38 (0.57, 3.31)	0.478
Only postnatal			1.13 (0.14, 9.21)	0.907	1.04 (0.12, 8.76)	0.971
1st sampling time (mo)			0.89 (0.72, 1.11)	0.308	1.08 (0.86, 1.37)	0.496
Maternal race/ethnicity (Ref.: White)						
Others					1.15 (0.57, 2.34)	0.690
Asian					3.03 (1.87, 4.92)	<0.001
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.00 (0.00, N.A.)	0.997
Maternal prenatal food allergy (Ref.: No)						
Yes					1.56 (1.01, 2.40)	0.044

Table S23. Multiple logistic regression for the association between food sensitization at age 1 year and late infancy microbiota clusters.

	Model 1 (BIC: 893.9)		Model 2 (BIC: 815.1)		Model 3 (BIC: 774.5)	
	Coefficient	95% CI	p	Coefficient	95% CI	p
Late infancy cluster membership (Ref.: C2)						
C1	1.78	(1.17, 2.70)	0.007	1.62	(1.01, 2.62)	0.046
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency				0.77	(0.42, 1.40)	0.391
Cesarean, scheduled				0.95	(0.47, 1.90)	0.882
Vaginal, IAP+				0.74	(0.44, 1.24)	0.255
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No				1.01	(0.57, 1.79)	0.982
Partial				1.07	(0.67, 1.71)	0.770
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes				0.85	(0.50, 1.44)	0.543
Siblings (Ref.: Yes)						
No				1.24	(0.82, 1.88)	0.303
Household pet exposure (Ref.: Both prenatal and postnatal)						
No				1.84	(1.20, 2.82)	0.005
Only prenatal				1.15	(0.46, 2.86)	0.765
Only postnatal				1.20	(0.15, 9.76)	0.863
2nd sampling time (mo)				0.77	(0.62, 0.96)	0.019
Maternal race/ethnicity (Ref.: White)						
Others					1.13	(0.56, 2.30)
Asian					2.94	(1.79, 4.83)
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.00	(0.00, N.A.)
Maternal prenatal food allergy (Ref.: No)						
Yes					1.58	(1.02, 2.45)

Table S24. Multiple logistic regression for the association between food sensitization at age 3 years and early infancy microbiota clusters.

	Model 1 (BIC: 585.0)		Model 2 (BIC: 577.8)		Model 3 (BIC: 578.5)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
Early infancy cluster membership (Ref.: C2)						
C1	1.35 (0.84, 2.17)	0.214	1.28 (0.74, 2.24)	0.377	1.17 (0.66, 2.07)	0.582
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			1.08 (0.52, 2.25)	0.840	1.04 (0.49, 2.20)	0.920
Cesarean, scheduled			1.10 (0.45, 2.70)	0.830	1.20 (0.48, 2.99)	0.699
Vaginal, IAP+			0.79 (0.40, 1.54)	0.481	0.80 (0.41, 1.59)	0.529
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.84 (0.39, 1.78)	0.648	0.89 (0.41, 1.92)	0.770
Partial			0.93 (0.51, 1.71)	0.814	0.86 (0.46, 1.62)	0.646
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			1.05 (0.56, 1.99)	0.876	1.02 (0.54, 1.96)	0.944
Siblings (Ref.: Yes)						
No			1.64 (0.96, 2.78)	0.069	1.77 (1.03, 3.06)	0.039
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			2.10 (1.21, 3.64)	0.009	1.86 (1.04, 3.33)	0.037
Only prenatal			0.99 (0.28, 3.45)	0.987	1.08 (0.30, 3.85)	0.905
Only postnatal			0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.999
1st sampling time (mo)			0.89 (0.66, 1.19)	0.419	1.01 (0.74, 1.39)	0.925
Maternal race/ethnicity (Ref.: White)						
Others					2.19 (1.04, 4.61)	0.040
Asian					2.87 (1.57, 5.25)	0.001
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.22 (0.03, 1.70)	0.146
Maternal prenatal food allergy (Ref.: No)						
Yes					0.75 (0.41, 1.38)	0.354

Table S25. Multiple logistic regression for the association between food sensitization at age 3 years and late infancy microbiota clusters.

	Model 1 (BIC: 576.2)		Model 2 (BIC: 558.2)		Model 3 (BIC: 563.3)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
Late infancy cluster membership (Ref.: C2)						
C1	2.42 (1.45, 4.02)	0.001	2.30 (1.31, 4.04)	0.004	1.83 (1.01, 3.31)	0.045
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			1.02 (0.50, 2.11)	0.946	0.96 (0.46, 2.02)	0.923
Cesarean, scheduled			1.19 (0.50, 2.84)	0.697	1.23 (0.51, 2.99)	0.642
Vaginal, IAP+			0.78 (0.39, 1.52)	0.458	0.79 (0.40, 1.57)	0.506
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.85 (0.40, 1.82)	0.670	0.88 (0.41, 1.92)	0.756
Partial			0.88 (0.47, 1.65)	0.681	0.80 (0.42, 1.54)	0.507
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			1.09 (0.57, 2.08)	0.789	1.07 (0.55, 2.05)	0.847
Siblings (Ref.: Yes)						
No			1.52 (0.89, 2.61)	0.129	1.68 (0.96, 2.92)	0.067
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			2.06 (1.18, 3.59)	0.011	1.85 (1.03, 3.33)	0.040
Only prenatal			0.66 (0.15, 2.92)	0.579	0.68 (0.15, 3.08)	0.618
Only postnatal			0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.999
2nd sampling time (mo)			0.76 (0.56, 1.02)	0.064	0.81 (0.60, 1.07)	0.139
Maternal race/ethnicity (Ref.: White)						
Others					2.12 (0.99, 4.50)	0.052
Asian					2.62 (1.40, 4.88)	0.002
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.26 (0.04, 1.98)	0.194
Maternal prenatal food allergy (Ref.: No)						
Yes					0.76 (0.41, 1.40)	0.378

Table S26. Multiple logistic regression for the association between inhalant sensitization at age 1 year and early infancy microbiota clusters.

	Model 1 (BIC: 419.9)		Model 2 (BIC: 410.2)		Model 3 (BIC: 412.0)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
Early infancy cluster membership (Ref.: C2)						
C1	0.90 (0.50, 1.62)	0.721	0.63 (0.30, 1.32)	0.225	0.66 (0.31, 1.43)	0.297
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			2.81 (1.07, 7.42)	0.037	2.97 (1.09, 8.12)	0.033
Cesarean, scheduled			3.23 (1.10, 9.44)	0.032	3.35 (1.11, 10.14)	0.032
Vaginal, IAP+			1.32 (0.55, 3.16)	0.539	1.45 (0.58, 3.58)	0.423
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.46 (0.15, 1.35)	0.157	0.51 (0.17, 1.57)	0.243
Partial			0.46 (0.19, 1.13)	0.090	0.47 (0.19, 1.17)	0.104
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			1.04 (0.46, 2.33)	0.932	1.16 (0.51, 2.63)	0.729
Siblings (Ref.: Yes)						
No			0.67 (0.33, 1.35)	0.262	0.61 (0.29, 1.28)	0.194
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			0.68 (0.35, 1.33)	0.261	0.53 (0.25, 1.09)	0.083
Only prenatal			0.00 (0.00, N.A.)	0.997	0.00 (0.00, N.A.)	0.997
Only postnatal			0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.999
1st sampling time (mo)			1.02 (0.74, 1.42)	0.891	1.14 (0.79, 1.65)	0.474
Maternal race/ethnicity (Ref.: White)						
Others					2.02 (0.73, 5.63)	0.177
Asian					3.47 (1.51, 8.01)	0.003
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.37 (0.04, 3.08)	0.357
Maternal prenatal food allergy (Ref.: No)						
Yes					1.56 (0.76, 3.22)	0.228

Table S27. Multiple logistic regression for the association between inhalant sensitization at age 1 year and late infancy microbiota clusters.

	Model 1 (BIC: 420.1)		Model 2 (BIC: 402.3)		Model 3 (BIC: 404.9)	
	Coefficient	95% CI	p	Coefficient	95% CI	p
Late infancy cluster membership (Ref.: C2)						
C1	1.03	(0.48, 2.25)	0.930	0.96	(0.39, 2.32)	0.922
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency				2.31	(0.92, 5.81)	0.074
Cesarean, scheduled				2.00	(0.69, 5.77)	0.200
Vaginal, IAP+				1.22	(0.51, 2.92)	0.662
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No				0.35	(0.10, 1.18)	0.091
Partial				0.53	(0.22, 1.25)	0.146
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes				1.10	(0.49, 2.47)	0.826
Siblings (Ref.: Yes)						
No				0.64	(0.31, 1.32)	0.229
Household pet exposure (Ref.: Both prenatal and postnatal)						
No				0.73	(0.37, 1.43)	0.355
Only prenatal				0.00	(0.00, N.A.)	0.997
Only postnatal				0.00	(0.00, N.A.)	0.999
2nd sampling time (mo)				0.76	(0.52, 1.10)	0.145
Maternal race/ethnicity (Ref.: White)						
Others					2.02	(0.72, 5.60)
Asian					3.17	(1.31, 7.68)
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.65	(0.08, 5.00)
Maternal prenatal food allergy (Ref.: No)						
Yes					1.44	(0.68, 3.04)

Table S28. Multiple logistic regression for the association between inhalant sensitization at age 3 years and early infancy microbiota clusters.

	Model 1 (BIC: 862.2)		Model 2 (BIC: 795.7)		Model 3 (BIC: 784.8)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
Early infancy cluster membership (Ref.: C2)						
C1	0.95 (0.66, 1.37)	0.788	0.82 (0.53, 1.28)	0.386	0.82 (0.52, 1.29)	0.392
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			1.66 (0.92, 2.98)	0.094	1.52 (0.83, 2.79)	0.171
Cesarean, scheduled			1.83 (0.95, 3.52)	0.070	1.66 (0.84, 3.29)	0.147
Vaginal, IAP+			0.78 (0.44, 1.37)	0.385	0.74 (0.42, 1.30)	0.295
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			1.16 (0.67, 2.01)	0.593	1.21 (0.68, 2.14)	0.516
Partial			1.06 (0.66, 1.71)	0.821	1.02 (0.63, 1.67)	0.925
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			1.34 (0.84, 2.16)	0.221	1.41 (0.87, 2.29)	0.168
Siblings (Ref.: Yes)						
No			0.90 (0.59, 1.38)	0.638	0.89 (0.57, 1.36)	0.581
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			1.23 (0.81, 1.88)	0.322	0.93 (0.60, 1.46)	0.756
Only prenatal			0.71 (0.27, 1.88)	0.495	0.69 (0.26, 1.84)	0.459
Only postnatal			3.12 (0.61, 15.92)	0.170	2.60 (0.48, 14.00)	0.265
1st sampling time (mo)			1.12 (0.92, 1.36)	0.249	1.25 (1.01, 1.56)	0.044
Maternal race/ethnicity (Ref.: White)						
Others					1.54 (0.80, 2.95)	0.193
Asian					3.97 (2.41, 6.52)	<0.001
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.62 (0.24, 1.59)	0.317
Maternal prenatal food allergy (Ref.: No)						
Yes					1.28 (0.81, 2.01)	0.289

Table S29. Multiple logistic regression for the association between inhalant sensitization at age 3 years and late infancy microbiota clusters.

	Model 1 (BIC: 859.1)		Model 2 (BIC: 767.9)		Model 3 (BIC: 766.6)	
	Coefficient	95% CI	p	Coefficient	95% CI	p
Late infancy cluster membership (Ref.: C2)						
C1	1.50	(0.97, 2.32)	0.069	1.70	(1.03, 2.81)	0.038
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency				1.48	(0.84, 2.61)	0.174
Cesarean, scheduled				1.56	(0.83, 2.94)	0.169
Vaginal, IAP+				0.73	(0.41, 1.29)	0.281
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No				1.33	(0.76, 2.33)	0.314
Partial				1.19	(0.73, 1.93)	0.490
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes				1.39	(0.86, 2.23)	0.179
Siblings (Ref.: Yes)						
No				0.85	(0.55, 1.31)	0.454
Household pet exposure (Ref.: Both prenatal and postnatal)						
No				1.21	(0.79, 1.85)	0.390
Only prenatal				0.56	(0.19, 1.64)	0.290
Only postnatal				3.39	(0.66, 17.39)	0.144
2nd sampling time (mo)				1.06	(0.89, 1.27)	0.508
Maternal race/ethnicity (Ref.: White)						
Others					1.57	(0.82, 3.00)
Asian					3.31	(1.98, 5.53)
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.88	(0.36, 2.16)
Maternal prenatal food allergy (Ref.: No)						
Yes					1.26	(0.80, 1.99)

Table S30. Multiple logistic regression for the association between peanut sensitization at age 3 years and early infancy microbiota clusters.

	Model 1 (BIC: 401.8)		Model 2 (BIC: 408.1)		Model 3 (BIC: 412.3)	
	Coefficient	95% CI	p	Coefficient	95% CI	p
Early infancy cluster membership (Ref.: C2)						
C1	1.63	(0.88, 3.04)	0.122	1.99	(0.94, 4.21)	0.072
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency				0.76	(0.30, 1.92)	0.561
Cesarean, scheduled				1.11	(0.39, 3.20)	0.847
Vaginal, IAP+				0.33	(0.11, 0.97)	0.043
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No				0.61	(0.21, 1.82)	0.381
Partial				1.00	(0.46, 2.16)	0.996
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes				0.80	(0.32, 1.95)	0.616
Siblings (Ref.: Yes)						
No				1.98	(0.99, 3.96)	0.055
Household pet exposure (Ref.: Both prenatal and postnatal)						
No				1.86	(0.91, 3.80)	0.087
Only prenatal				0.96	(0.21, 4.48)	0.963
Only postnatal				0.00	(0.00, .)	0.999
1st sampling time (mo)				0.89	(0.61, 1.31)	0.562
Maternal race/ethnicity (Ref.: White)						
Others					2.80	(1.06, 7.38)
Asian					4.10	(1.91, 8.82)
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.47	(0.06, 3.95)
Maternal prenatal food allergy (Ref.: No)						
Yes					1.06	(0.50, 2.23)

Table S31. Multiple logistic regression for the association between peanut sensitization at age 3 years and late infancy microbiota clusters.

	Model 1 (BIC: 395.2)		Model 2 (BIC: 393.5)		Model 3 (BIC: 400.5)				
	Coefficient	95% CI	p	Coefficient	95% CI	p			
Late infancy cluster membership (Ref.: C2)									
C1	2.78	(1.48, 5.22)	0.001	2.82	(1.38, 5.76)	0.004	2.02	(0.95, 4.32)	0.069
Birth mode (Ref.: Vaginal, IAP−)									
Cesarean, emergency				0.80	(0.32, 2.01)	0.636	0.75	(0.29, 1.95)	0.560
Cesarean, scheduled				1.46	(0.52, 4.14)	0.476	1.56	(0.54, 4.54)	0.412
Vaginal, IAP+				0.34	(0.11, 1.00)	0.051	0.34	(0.11, 1.03)	0.057
Breastfeeding status (0-3 mo) (Ref.: Exclusive)									
No				0.60	(0.20, 1.80)	0.362	0.64	(0.21, 1.96)	0.437
Partial				0.86	(0.38, 1.94)	0.717	0.73	(0.31, 1.73)	0.479
Direct antibiotic exposure (0-12 mo) (Ref.: No)									
Yes				0.83	(0.34, 2.06)	0.691	0.86	(0.34, 2.17)	0.753
Siblings (Ref.: Yes)									
No				1.91	(0.93, 3.89)	0.076	2.18	(1.03, 4.58)	0.041
Household pet exposure (Ref.: Both prenatal and postnatal)									
No				1.83	(0.89, 3.74)	0.098	1.50	(0.69, 3.26)	0.301
Only prenatal				0.46	(0.06, 3.72)	0.468	0.43	(0.05, 3.58)	0.433
Only postnatal				0.00	(0.00, N.A.)	0.999	0.00	(0.00, N.A.)	0.999
2nd sampling time (mo)				0.82	(0.56, 1.19)	0.287	0.86	(0.60, 1.24)	0.420
Maternal race/ethnicity (Ref.: White)									
Others							2.72	(1.01, 7.28)	0.047
Asian							3.82	(1.73, 8.44)	0.001
Solid food introduction (0-3 mo) (Ref.: No)									
Yes							0.54	(0.07, 4.32)	0.565
Maternal prenatal food allergy (Ref.: No)									
Yes							1.11	(0.52, 2.35)	0.791

Table S32. Mixed effect regression for the association between peanut sensitization at age 3 years and prevalent genera or the E/B ratio.

Genus	Prevalence		Model 1		Model 2		Model 3	
	Early infancy	Late infancy	Coefficient 95% CI	p value	Coefficient 95% CI	p value	Coefficient 95% CI	p value
EB_ratio	N.A.	N.A.	0.06 (0.106, 0.014)	0.011	0.059 (0.105, 0.012)	0.013	0.042 (0.09, -0.005)	0.08
Streptococcus	0.994	0.994	0.004 (-0.014, 0.021)	0.677	-0.003 (-0.022, 0.016)	0.759	-0.008 (-0.027, 0.012)	0.439
Bacteroides	0.992	0.999	-0.107 (-0.2, -0.013)	0.025	-0.097 (-0.192, -0.003)	0.044	-0.081 (-0.178, 0.015)	0.099
Veillonella	0.991	0.997	0.04 (-0.002, 0.082)	0.062	0.032 (-0.011, 0.075)	0.145	0.024 (-0.02, 0.068)	0.287
Unclassified Enterobacteriaceae	0.998	0.986	0.077 (0.027, 0.127)	0.003	0.086 (0.033, 0.138)	0.001	0.058 (0.005, 0.111)	0.033
Bifidobacterium	0.977	0.990	-0.009 (-0.054, 0.035)	0.685	-0.018 (-0.066, 0.03)	0.460	-0.02 (-0.069, 0.029)	0.423
Unclassified Lachnospiraceae	0.797	0.999	-0.009 (-0.041, 0.024)	0.596	-0.008 (-0.04, 0.024)	0.620	0.003 (-0.029, 0.036)	0.846
Unclassified Clostridiaceae	0.785	0.987	0.016 (-0.006, 0.038)	0.148	0.016 (-0.006, 0.039)	0.160	0.012 (-0.012, 0.035)	0.327
Clostridium (Clostridiaceae)	0.706	0.735	0.039 (0.012, 0.067)	0.005	0.042 (0.013, 0.071)	0.005	0.027 (-0.002, 0.057)	0.069
.Ruminococcus.	0.686	0.990	0.001 (-0.024, 0.026)	0.935	0.008 (-0.02, 0.035)	0.574	0.011 (-0.017, 0.039)	0.451
Haemophilus	0.678	0.919	-0.01 (-0.026, 0.005)	0.192	-0.013 (-0.029, 0.004)	0.124	-0.015 (-0.032, 0.002)	0.080
Actinomyces	0.684	0.628	0.002 (-0.004, 0.007)	0.536	0.001 (-0.005, 0.007)	0.788	0.001 (-0.005, 0.007)	0.669
Unclassified Gemellaceae	0.520	0.539	-0.001 (-0.004, 0.002)	0.590	-0.001 (-0.005, 0.002)	0.423	-0.001 (-0.005, 0.002)	0.449
Oscillospira	0.518	0.973	0.012 (-0.005, 0.029)	0.162	0.008 (-0.009, 0.025)	0.348	0.01 (-0.007, 0.027)	0.255
Parabacteroides	0.513	0.660	-0.012 (-0.044, 0.02)	0.459	-0.015 (-0.048, 0.018)	0.378	-0.012 (-0.045, 0.022)	0.496
Unclassified Erysipelotrichaceae	0.477	0.974	0.009 (-0.005, 0.024)	0.202	0.012 (-0.004, 0.027)	0.135	0.014 (-0.002, 0.03)	0.080
Eggerthella	0.447	0.828	0.001 (-0.003, 0.005)	0.564	0.002 (-0.003, 0.006)	0.440	0.002 (-0.002, 0.006)	0.367
Citrobacter	0.703	0.437	0.003 (-0.004, 0.01)	0.412	0.001 (-0.006, 0.009)	0.743	-0.001 (-0.009, 0.007)	0.858
Unclassified Lachnospiraceae (Other)	0.429	0.945	-0.003 (-0.012, 0.006)	0.529	-0.002 (-0.012, 0.008)	0.691	0.001 (-0.009, 0.011)	0.874
Enterococcus	0.669	0.412	0.001 (-0.005, 0.006)	0.776	0.001 (-0.005, 0.007)	0.862	0 (-0.006, 0.006)	0.935
Lactobacillus	0.402	0.380	-0.005 (-0.012, 0.002)	0.161	-0.005 (-0.013, 0.002)	0.140	-0.005 (-0.012, 0.002)	0.174
Ruminococcus	0.380	0.911	-0.007 (-0.018, 0.004)	0.225	-0.004 (-0.015, 0.008)	0.527	-0.001 (-0.013, 0.01)	0.825
Blautia	0.357	0.940	-0.002 (-0.018, 0.013)	0.753	-0.003 (-0.019, 0.014)	0.740	0.001 (-0.015, 0.018)	0.883
Unclassified Lactobacillales (Other)	0.338	0.657	0 (-0.003, 0.003)	0.871	0 (-0.003, 0.003)	0.870	0 (-0.003, 0.004)	0.873
Prevotella	0.330	0.658	-0.011 (-0.039, 0.016)	0.426	-0.011 (-0.042, 0.02)	0.486	-0.01 (-0.043, 0.022)	0.524
Akkermansia	0.318	0.679	-0.016 (-0.048, 0.016)	0.336	-0.013 (-0.047, 0.022)	0.473	NA	NA
Megasphaera	0.309	0.613	0.003 (-0.015, 0.021)	0.746	0.005 (-0.015, 0.026)	0.597	0.002 (-0.019, 0.023)	0.866
Epulopiscium	0.307	0.385	-0.005 (-0.017, 0.008)	0.461	-0.007 (-0.02, 0.007)	0.313	-0.007 (-0.021, 0.007)	0.304
Sutterella	0.297	0.785	-0.023 (-0.044, -0.002)	0.029	-0.019 (-0.042, 0.003)	0.093	-0.013 (-0.036, 0.011)	0.289
Unclassified Ruminococcaceae	0.277	0.914	-0.019 (-0.038, -0.001)	0.044	-0.017 (-0.037, 0.003)	0.100	-0.011 (-0.032, 0.009)	0.282
.Eubacterium.	0.275	0.878	0.009 (0.003, 0.016)	0.006	0.008 (0.002, 0.014)	0.014	0.007 (0.001, 0.014)	0.028
Unclassified Peptostreptococcaceae	0.262	0.326	0.004 (0.001, 0.007)	0.005	0.005 (0.001, 0.008)	0.004	0.004 (0.001, 0.007)	0.019
Dorea	0.252	0.821	0.008 (-0.003, 0.02)	0.161	0.006 (-0.006, 0.017)	0.348	0.004 (-0.007, 0.016)	0.463
Unclassified Enterobacteriaceae (Other)	0.674	0.252	0.004 (0, 0.008)	0.066	0.004 (0, 0.009)	0.074	0.003 (-0.002, 0.008)	0.191
Rothia	0.628	0.250	-0.002 (-0.006, 0.003)	0.478	-0.002 (-0.007, 0.002)	0.376	NA	NA
Trabulsiella	0.517	0.250	0.003 (-0.004, 0.011)	0.355	0.002 (-0.006, 0.011)	0.552	-0.001 (-0.009, 0.008)	0.873
Dialister	0.226	0.605	-0.009 (-0.024, 0.007)	0.286	-0.012 (-0.029, 0.004)	0.152	-0.01 (-0.027, 0.007)	0.258
Collinsella	0.212	0.428	0.005 (-0.008, 0.017)	0.494	0.009 (-0.005, 0.023)	0.212	0.011 (-0.003, 0.025)	0.125

Unclassified									
Clostridiales	0.209	0.942	-0.012 (-0.031, 0.007)	0.224	-0.015 (-0.035, 0.006)	0.163	-0.009 (-0.03, 0.012)	0.389	

Relative abundance data was arcsine square root transformed for modelling, and coefficients (95% CI) shown here are in arcsine square root scale.

* Ratio of Unclassified Enterobacteriaceae/*Bacteroides*.

Table S33. Mixed effect regression for the association between food sensitization at age 3 years and prevalent genera or the E/B ratio.

Genus	Prevalence		Model 1			Model 2			Model 3		
	Early infancy	Late infancy	Coefficient	95% CI	<i>p</i> value	Coefficient	95% CI	<i>p</i> value	Coefficient	95% CI	<i>p</i> value
EB_ratio	N.A.	N.A.	0.043 (0.079, 0.007)		0.02	0.03 (0.066, -0.006)		0.098	0.018 (0.055, -0.018)		0.322
Streptococcus	0.994	0.994	0.004 (-0.009, 0.018)		0.527	0.001 (-0.014, 0.015)		0.925	-0.002 (-0.017, 0.013)		0.780
Bacteroides	0.992	0.999	-0.078 (-0.151, -0.005)		0.036	-0.059 (-0.132, 0.014)		0.114	-0.048 (-0.122, 0.026)		0.203
Veillonella	0.991	0.997	0.025 (-0.008, 0.058)		0.138	0.017 (-0.016, 0.05)		0.322	0.012 (-0.022, 0.046)		0.482
Unclassified Enterobacteriaceae	0.998	0.986	0.06 (0.021, 0.1)		0.003	0.057 (0.017, 0.097)		0.006	0.037 (-0.004, 0.077)		0.073
Bifidobacterium	0.977	0.990	0.016 (-0.019, 0.051)		0.360	0.008 (-0.029, 0.045)		0.666	0.009 (-0.029, 0.046)		0.655
Unclassified Lachnospiraceae	0.797	0.999	-0.013 (-0.038, 0.012)		0.307	-0.011 (-0.036, 0.013)		0.368	-0.002 (-0.027, 0.023)		0.856
Unclassified Clostridiaceae	0.785	0.987	0.01 (-0.007, 0.027)		0.271	0.01 (-0.008, 0.027)		0.280	0.006 (-0.012, 0.024)		0.503
Clostridium (Clostridiaceae)	0.706	0.735	0.011 (-0.011, 0.032)		0.318	0.009 (-0.013, 0.031)		0.433	-0.001 (-0.024, 0.021)		0.907
.Ruminococcus.	0.686	0.990	-0.008 (-0.028, 0.012)		0.418	-0.002 (-0.023, 0.019)		0.829	0.001 (-0.02, 0.022)		0.926
Haemophilus	0.678	0.919	0.002 (-0.01, 0.015)		0.700	0.002 (-0.011, 0.014)		0.812	0 (-0.013, 0.013)		0.970
Actinomyces	0.684	0.628	0.003 (-0.002, 0.007)		0.231	0.002 (-0.003, 0.006)		0.483	0.002 (-0.003, 0.007)		0.388
Unclassified Gemellaceae	0.520	0.539	0 (-0.002, 0.003)		0.895	0 (-0.003, 0.002)		0.981	0 (-0.003, 0.003)		0.995
Oscillospira	0.518	0.973	-0.003 (-0.016, 0.011)		0.711	-0.005 (-0.018, 0.008)		0.486	-0.002 (-0.016, 0.011)		0.711
Parabacteroides	0.513	0.660	-0.008 (-0.033, 0.017)		0.517	-0.008 (-0.033, 0.018)		0.547	-0.007 (-0.033, 0.019)		0.614
Unclassified Erysipelotrichaceae	0.477	0.974	-0.001 (-0.012, 0.01)		0.842	0 (-0.012, 0.012)		0.970	0.001 (-0.011, 0.014)		0.811
Eggerthella	0.447	0.828	0.001 (-0.002, 0.004)		0.508	0.002 (-0.002, 0.005)		0.362	0.002 (-0.001, 0.005)		0.263
Citrobacter	0.703	0.437	0 (-0.006, 0.006)		0.993	-0.003 (-0.009, 0.003)		0.353	-0.004 (-0.01, 0.002)		0.148
Unclassified Lachnospiraceae (Other)	0.429	0.945	-0.004 (-0.011, 0.003)		0.248	-0.004 (-0.011, 0.004)		0.341	-0.002 (-0.01, 0.005)		0.574
Enterococcus	0.669	0.412	0 (-0.005, 0.004)		0.835	-0.002 (-0.007, 0.002)		0.327	-0.003 (-0.008, 0.002)		0.232
Lactobacillus	0.402	0.380	0.002 (-0.003, 0.008)		0.444	0 (-0.005, 0.006)		0.982	0 (-0.005, 0.006)		0.869
Ruminococcus	0.380	0.911	-0.01 (-0.018, -0.001)		0.023	-0.007 (-0.016, 0.001)		0.090	-0.006 (-0.014, 0.003)		0.192
Blautia	0.357	0.940	0.001 (-0.011, 0.012)		0.928	0.002 (-0.01, 0.015)		0.720	0.005 (-0.008, 0.018)		0.428
Unclassified Lactobacillales (Other)	0.338	0.657	0.001 (-0.002, 0.003)		0.658	0.001 (-0.001, 0.003)		0.402	0.001 (-0.001, 0.004)		0.401
Prevotella	0.330	0.658	0.002 (-0.02, 0.023)		0.881	0.003 (-0.02, 0.027)		0.775	0.004 (-0.021, 0.028)		0.777
Akkermansia	0.318	0.679	-0.01 (-0.035, 0.015)		0.441	-0.006 (-0.033, 0.02)		0.645	0.001 (-0.026, 0.028)		0.967
Megasphaera	0.309	0.613	0 (-0.014, 0.015)		0.946	0.001 (-0.014, 0.017)		0.881	-0.001 (-0.017, 0.015)		0.889
Epulopiscium	0.307	0.385	0 (-0.009, 0.01)		0.966	-0.002 (-0.012, 0.008)		0.697	-0.002 (-0.013, 0.009)		0.712
Sutterella	0.297	0.785	-0.016 (-0.032, 0)		0.050	-0.013 (-0.03, 0.005)		0.151	-0.008 (-0.026, 0.01)		0.369
Unclassified Ruminococcaceae	0.277	0.914	-0.016 (-0.031, -0.001)		0.039	-0.012 (-0.027, 0.004)		0.135	-0.008 (-0.023, 0.008)		0.324
.Eubacterium.	0.275	0.878	0.004 (-0.002, 0.009)		0.179	0.003 (-0.002, 0.008)		0.279	0.002 (-0.003, 0.007)		0.340
Unclassified Peptostreptococcaceae	0.262	0.326	0.002 (0, 0.005)		0.042	0.003 (0, 0.005)		0.042	0.002 (0, 0.005)		0.113
Dorea	0.252	0.821	0.002 (-0.007, 0.01)		0.737	0.001 (-0.008, 0.009)		0.905	0 (-0.009, 0.009)		0.961
Unclassified Enterobacteriaceae (Other)	0.674	0.252	0.002 (-0.001, 0.006)		0.160	0.002 (-0.001, 0.005)		0.261	0.001 (-0.002, 0.005)		0.532
Rothia	0.628	0.250	0 (-0.003, 0.003)		0.993	0 (-0.004, 0.003)		0.936	-0.001 (-0.004, 0.003)		0.644
Trabulsiella	0.517	0.250	0.001 (-0.005, 0.007)		0.697	0 (-0.006, 0.006)		0.934	-0.002 (-0.009, 0.004)		0.460
Dialister	0.226	0.605	-0.008 (-0.02, 0.005)		0.229	-0.01 (-0.023, 0.002)		0.114	-0.009 (-0.022, 0.004)		0.178

Collinsella	0.212	0.428	-0.002 (-0.012, 0.008)	0.713	0.001 (-0.01, 0.012)	0.868	0.002 (-0.009, 0.013)	0.724
Unclassified							-0.003 (-0.019,	
Clostridiales	0.209	0.942	-0.006 (-0.021, 0.009)	0.431	-0.006 (-0.022, 0.01)	0.440	0.013)	0.727

Relative abundance data was arcsine square root transformed for modelling, and coefficients (95% CI) shown here are in arcsine square root scale.

* Ratio of Unclassified Enterobacteriaceae/*Bacteroides*.

Table S34. Multiple logistic regression for the association between food sensitization at age 3 years and microbiota developmental trajectories together with ethnicity.

	Model 1 (BIC: 569.7)		Model 2 (BIC: 560.3)		Model 3 (BIC: 564.6)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
Maternal ethnicity and trajectory (Ref.: Non-Asian, non-C1-C1)						
Asian, C1-C1	5.99 (2.79, 12.87)	<0.001	5.72 (2.46, 13.26)	<0.001	4.67 (1.94, 11.21)	0.001
non-Asian, C1-C1	2.11 (1.10, 4.05)	0.024	2.10 (1.03, 4.30)	0.042	2.04 (0.99, 4.19)	0.052
Asian, non-C1-C1	2.55 (1.27, 5.14)	0.009	2.58 (1.22, 5.46)	0.013	2.51 (1.19, 5.31)	0.016
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			0.97 (0.46, 2.04)	0.937	0.98 (0.47, 2.07)	0.961
Cesarean, scheduled			1.27 (0.53, 3.05)	0.598	1.33 (0.55, 3.22)	0.527
Vaginal, IAP+			0.81 (0.41, 1.61)	0.557	0.85 (0.43, 1.70)	0.655
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.88 (0.40, 1.89)	0.734	0.91 (0.42, 1.97)	0.800
Partial			0.93 (0.49, 1.76)	0.826	0.88 (0.46, 1.68)	0.695
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			1.16 (0.60, 2.22)	0.657	1.12 (0.58, 2.16)	0.732
Siblings (Ref.: Yes)						
No			1.48 (0.85, 2.56)	0.167	1.60 (0.92, 2.81)	0.097
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			1.74 (0.98, 3.10)	0.061	1.90 (1.06, 3.42)	0.032
Only prenatal			0.65 (0.14, 2.92)	0.570	0.74 (0.16, 3.37)	0.700
Only postnatal			0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.999
1st sampling time (mo)			0.99 (0.72, 1.37)	0.972	1.07 (0.77, 1.49)	0.666
2nd sampling time (mo)			0.77 (0.57, 1.04)	0.089	0.79 (0.59, 1.07)	0.127
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.28 (0.04, 2.17)	0.221
Maternal prenatal food allergy (Ref.: No)						
Yes					0.73 (0.39, 1.37)	0.324

Table S35. Multiple logistic regression for the association between peanut sensitization at age 3 years and microbiota developmental trajectories together with ethnicity.

	Model 1 (BIC: 395.1)		Model 2 (BIC: 399.6)		Model 3 (BIC: 407.0)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
Maternal ethnicity and trajectory (Ref.: Non-Asian, non-C1-C1)						
Asian, C1-C1	8.61 (3.60, 20.55)	<0.001	9.78 (3.60, 26.59)	<0.001	7.87 (2.75, 22.55)	<0.001
non-Asian, C1-C1	2.31 (1.01, 5.30)	0.047	2.52 (0.99, 6.47)	0.054	2.38 (0.93, 6.11)	0.071
Asian, non-C1-C1	2.88 (1.20, 6.91)	0.018	3.27 (1.26, 8.51)	0.015	3.11 (1.19, 8.12)	0.020
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			0.68 (0.26, 1.79)	0.435	0.70 (0.27, 1.86)	0.479
Cesarean, scheduled			1.56 (0.55, 4.48)	0.405	1.70 (0.59, 4.89)	0.325
Vaginal, IAP+			0.34 (0.11, 1.01)	0.052	0.35 (0.12, 1.06)	0.065
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.61 (0.20, 1.85)	0.387	0.64 (0.21, 1.95)	0.433
Partial			0.95 (0.42, 2.15)	0.900	0.82 (0.35, 1.93)	0.657
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			0.89 (0.35, 2.22)	0.800	0.92 (0.36, 2.31)	0.855
Siblings (Ref.: Yes)						
No			1.91 (0.92, 3.95)	0.082	2.13 (1.01, 4.48)	0.047
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			1.45 (0.69, 3.08)	0.329	1.64 (0.76, 3.54)	0.209
Only prenatal			0.41 (0.05, 3.46)	0.411	0.45 (0.05, 3.86)	0.467
Only postnatal			0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.999
1st sampling time (mo)			1.01 (0.67, 1.52)	0.977	1.07 (0.69, 1.66)	0.768
2nd sampling time (mo)			0.83 (0.57, 1.20)	0.317	0.85 (0.58, 1.23)	0.393
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.60 (0.07, 5.13)	0.641
Maternal prenatal food allergy (Ref.: No)						
Yes					1.16 (0.55, 2.47)	0.699

Table S36: Modification of the effect of C1-C1 trajectory on food sensitization at age 3 by Asian genetic background

		Non C1-C1		C1-C1		OR (95%) for C1-C1 within strata of Ethnicity
	N with/without Food Sensitization	OR (95% CI); P		N with/without Food Sensitization	OR (95% CI); P	
Non-Asian	32/736	1		12/114	2.04 (0.99, 4.19) p = 0.052	2.14 (1.03, 4.45) p = 0.042
Asian	11/82	2.51 (1.19, 5.31) p = 0.016		9/31	4.67 (1.94, 11.21) p = 0.001	2.06 (0.59, 7.17) p = 0.257

Measure of effect modification on additive scale (RERI): 1.43 (-2.92, 7.40)

Measure of effect modification on multiplicative scale: 0.91 (0.27, 3.08), p = 0.881.

ORs are adjusted for birth mode, breastfeeding status in the first 3 months, pet exposure, antibiotic exposure in the first year, having siblings, and sampling time.
Confidence interval of the additive scales was generated using 1000 times bootstrapping.

Table S37: Modification of the effect of C1-C1 trajectory on peanut sensitization at age 3 by Asian genetic background

		Non C1-C1		C1-C1		OR (95%) for C1-C1 within strata of Ethnicity
	N with/without Food Sensitization	OR (95% CI); P		N with/without Food Sensitization	OR (95% CI); P	
Non-Asian	16/749	1		7/119	2.38 (0.93, 6.11) p = 0.071	2.39 (0.91, 6.27) p = 0.076
Asian	7/86	3.11 (1.19, 8.12) p = 0.020		7/32	7.87 (2.75, 22.55) p < 0.001	3.79 (0.87, 16.52) p = 0.076

Measure of effect modification on additive scale (RERI): 4.50 (-3.80, 19.50)

Measure of effect modification on multiplicative scale: 1.06 (0.24, 4.70), p = 0.938.

ORs are adjusted for birth mode, breastfeeding status in the first 3 months, pet exposure, antibiotic exposure in the first year, having siblings, and sampling time.

Confidence interval of the additive scale was generated using 1000 times bootstrapping.

Table S38. Analysis of a causal mechanism in the association between Asian ethnicity and food sensitization at age 3.

	Estimate	95% CI Lower	95% CI Upper	p-value
Total effect	0.076	0.022	0.130	0.004
ACME	0.011	0	0.020	0.038
ADE	0.065	0.015	0.120	0.022
Prop. Mediated	0.145	0.004	0.390	0.042

ACME: average casual mediation effect; ADE: average direct effect. 95% CI generated using 1000 bootstraps.

Table S39. Analysis of a causal mechanism in the association between Asian ethnicity and food sensitization at age 3.

	Estimate	95% CI Lower	95% CI Upper	p-value
Total effect	0.062	0.017	0.120	0.006
ACME	0.007	0	0.020	0.048
ADE	0.055	0.011	0.110	0.012
Prop. Mediated	0.111	-0.002	0.420	0.054

ACME: average casual mediation effect; ADE: average direct effect. 95% CI generated using 1000 bootstraps.

Table S40. Multiple logistic regression for the association between food sensitization at age 3 years and *C. difficile* colonization in the first year.

	Model 1 (BIC: 520.4)		Model 2 (BIC: 516.5)		Model 3 (BIC: 526.3)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
C. difficile targeted PCR result (Ref.: A-A)						
A-P	1.29 (0.68, 2.46)	0.440	1.25 (0.63, 2.49)	0.517	1.18 (0.59, 2.38)	0.635
P-P	1.54 (0.73, 3.27)	0.259	1.48 (0.63, 3.50)	0.367	1.64 (0.69, 3.93)	0.265
P-A	1.87 (0.93, 3.76)	0.080	1.72 (0.76, 3.86)	0.191	2.08 (0.90, 4.81)	0.086
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			1.30 (0.61, 2.75)	0.495	1.12 (0.52, 2.42)	0.773
Cesarean, scheduled			1.58 (0.65, 3.88)	0.313	1.53 (0.61, 3.80)	0.361
Vaginal, IAP+			0.71 (0.33, 1.51)	0.372	0.68 (0.31, 1.47)	0.323
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.75 (0.33, 1.68)	0.482	0.75 (0.33, 1.72)	0.501
Partial			0.74 (0.38, 1.47)	0.389	0.76 (0.39, 1.52)	0.441
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			1.33 (0.69, 2.59)	0.396	1.26 (0.64, 2.50)	0.501
Siblings (Ref.: Yes)						
No			1.93 (1.07, 3.46)	0.028	2.04 (1.12, 3.69)	0.020
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			2.51 (1.36, 4.64)	0.003	2.12 (1.12, 4.01)	0.021
Only prenatal			0.86 (0.19, 3.88)	0.841	0.85 (0.18, 3.92)	0.837
Only postnatal			0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.999
1st sampling time (mo)			0.96 (0.69, 1.34)	0.802	1.02 (0.72, 1.46)	0.891
2nd sampling time (mo)			0.71 (0.51, 1.00)	0.052	0.75 (0.54, 1.04)	0.082
Maternal race/ethnicity (Ref.: White)						
Others					1.89 (0.82, 4.38)	0.136
Asian					3.01 (1.52, 5.97)	0.002
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.27 (0.03, 2.17)	0.219
Maternal prenatal food allergy (Ref.: No)						
Yes					0.64 (0.32, 1.26)	0.192

Table S41. Multiple logistic regression for the association between food sensitization at age 3 years and *C. difficile* colonization in the first year together with microbiota developmental trajectories.

	Model 1 (BIC: 527.9)		Model 2 (BIC: 527.3)		Model 3 (BIC: 538.1)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
Trajectory and <i>C. difficile</i> colonization (Ref.: Non-C1-C1, A-A)						
C1-C1, A-P	3.91 (1.52, 10.08)	0.005	3.54 (1.27, 9.87)	0.016	2.70 (0.93, 7.82)	0.067
C1-C1, P-P	4.86 (1.64, 14.39)	0.004	4.74 (1.40, 16.03)	0.012	5.69 (1.62, 19.99)	0.007
C1-C1, P-A	1.31 (0.17, 10.44)	0.797	1.22 (0.14, 10.45)	0.857	1.19 (0.13, 10.67)	0.879
C1-C1, A-A	2.26 (0.79, 6.46)	0.127	1.91 (0.63, 5.80)	0.255	1.71 (0.56, 5.25)	0.350
Non-C1-C1, A-P	1.11 (0.50, 2.45)	0.802	1.06 (0.45, 2.50)	0.887	1.05 (0.44, 2.50)	0.909
Non-C1-C1, P-P	1.23 (0.47, 3.24)	0.673	1.14 (0.38, 3.46)	0.815	1.23 (0.40, 3.81)	0.713
Non-C1-C1, P-A	2.39 (1.11, 5.14)	0.026	2.25 (0.92, 5.52)	0.076	2.81 (1.11, 7.09)	0.029
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			1.06 (0.48, 2.32)	0.884	0.93 (0.42, 2.07)	0.858
Cesarean, scheduled			1.65 (0.66, 4.07)	0.282	1.55 (0.61, 3.90)	0.353
Vaginal, IAP+			0.72 (0.34, 1.56)	0.410	0.68 (0.31, 1.48)	0.329
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.76 (0.33, 1.74)	0.519	0.74 (0.32, 1.73)	0.491
Partial			0.81 (0.41, 1.64)	0.564	0.84 (0.42, 1.70)	0.631
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			1.36 (0.69, 2.67)	0.376	1.32 (0.66, 2.62)	0.433
Siblings (Ref.: Yes)						
No			1.72 (0.94, 3.15)	0.081	1.85 (1.00, 3.43)	0.050
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			2.36 (1.27, 4.39)	0.006	2.06 (1.08, 3.92)	0.028
Only prenatal			0.80 (0.17, 3.72)	0.775	0.74 (0.15, 3.59)	0.710
Only postnatal			0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.999
1st sampling time (mo)			0.92 (0.65, 1.31)	0.659	0.98 (0.68, 1.41)	0.916
2nd sampling time (mo)			0.74 (0.52, 1.04)	0.086	0.76 (0.55, 1.06)	0.111
Maternal race/ethnicity (Ref.: White)						
Others					1.76 (0.73, 4.28)	0.210
Asian					2.91 (1.44, 5.86)	0.003
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.29 (0.04, 2.39)	0.250
Maternal prenatal food allergy (Ref.: No)						
Yes					0.58 (0.28, 1.18)	0.132

Table S42. Multiple logistic regression for the association between peanut sensitization at age 3 years and *C. difficile* colonization in the first year together with microbiota developmental trajectories.

	Model 1 (BIC: 370.1)		Model 2 (BIC: 384.3)		Model 3 (BIC: 398.8)	
	Coefficient 95% CI	p	Coefficient 95% CI	p	Coefficient 95% CI	p
Trajectory and <i>C. difficile</i> colonization (Ref.: Non-C1-C1, A-A)						
C1-C1, A-P	5.83 (1.78, 19.08)	0.004	4.49 (1.26, 16.03)	0.021	3.68 (0.98, 13.85)	0.054
C1-C1, P-P	6.12 (1.50, 24.97)	0.012	4.71 (0.99, 22.46)	0.052	5.89 (1.16, 29.87)	0.032
C1-C1, P-A	2.86 (0.34, 24.35)	0.337	2.32 (0.25, 21.77)	0.461	2.19 (0.21, 22.73)	0.510
C1-C1, A-A	2.86 (0.72, 11.35)	0.136	1.99 (0.47, 8.38)	0.350	1.78 (0.41, 7.67)	0.436
Non-C1-C1, A-P	1.75 (0.62, 4.87)	0.288	1.38 (0.47, 4.06)	0.558	1.37 (0.46, 4.13)	0.575
Non-C1-C1, P-P	1.32 (0.34, 5.17)	0.692	0.75 (0.14, 3.98)	0.738	0.83 (0.15, 4.42)	0.825
Non-C1-C1, P-A	2.69 (0.93, 7.78)	0.069	1.62 (0.44, 5.99)	0.473	2.14 (0.55, 8.29)	0.269
Birth mode (Ref.: Vaginal, IAP-)						
Cesarean, emergency			0.96 (0.36, 2.55)	0.929	0.78 (0.28, 2.17)	0.633
Cesarean, scheduled			2.12 (0.72, 6.28)	0.175	2.10 (0.70, 6.33)	0.186
Vaginal, IAP+			0.20 (0.05, 0.92)	0.038	0.19 (0.04, 0.84)	0.029
Breastfeeding status (0-3 mo) (Ref.: Exclusive)						
No			0.60 (0.18, 1.93)	0.389	0.60 (0.18, 1.97)	0.402
Partial			0.88 (0.36, 2.16)	0.787	0.90 (0.37, 2.21)	0.818
Direct antibiotic exposure (0-12 mo) (Ref.: No)						
Yes			1.01 (0.39, 2.61)	0.987	0.96 (0.36, 2.54)	0.936
Siblings (Ref.: Yes)						
No			2.33 (1.03, 5.24)	0.042	2.50 (1.09, 5.77)	0.031
Household pet exposure (Ref.: Both prenatal and postnatal)						
No			2.28 (1.01, 5.16)	0.048	1.81 (0.77, 4.30)	0.177
Only prenatal			0.56 (0.06, 4.78)	0.592	0.42 (0.05, 3.81)	0.438
Only postnatal			0.00 (0.00, N.A.)	0.999	0.00 (0.00, N.A.)	0.999
1st sampling time (mo)			0.99 (0.63, 1.56)	0.978	1.03 (0.64, 1.67)	0.891
2nd sampling time (mo)			0.72 (0.45, 1.16)	0.178	0.76 (0.48, 1.18)	0.216
Maternal race/ethnicity (Ref.: White)						
Others					3.81 (1.35, 10.70)	0.011
Asian					3.55 (1.42, 8.91)	0.007
Solid food introduction (0-3 mo) (Ref.: No)						
Yes					0.61 (0.07, 5.62)	0.663
Maternal prenatal food allergy (Ref.: No)						
Yes					0.92 (0.40, 2.15)	0.852

Table S43. Median (interquartile range) values of fecal metabolite concentration in different groups.

	Early infancy				Late infancy			
	C1-C1 (N = 67)	C1-C2 (N = 166)	C2-C2 (N = 268)	p value*	C1-C1 (N = 29)	C1-C2 (N = 56)	C2-C2 (N = 92)	p value*
Acetate	80 (47.3, 117.2)	71.5 (41.6, 110.9)	71.2 (43.4, 115.7)	0.560	58.9 (48.4, 78.8) ^a	53 (42.8, 66.2) ^{ab}	48.7 (38.5, 64.3) ^b	0.043
Butyrate	5.5 (1.5, 10.9)	4.7 (0.9, 11.8)	3.8 (1.1, 9.8)	0.614	11.7 (6.4, 15.2)	11 (6.9, 19.5)	10.5 (6.7, 15.4)	0.630
Propionate	10.2 (4.5, 15.8) ^{ab}	7.4 (2.5, 13.9) ^a	14 (4.4, 24.3) ^b	<0.001	7.3 (5.7, 12.3) ^a	14.8 (10.5, 21.4) ^b	15.2 (9.9, 23.1) ^b	<0.001
Valerate	0.4 (0, 1.3)	0.3 (0, 1.7)	0.4 (0, 1.5)	0.586	0.4 (0.3, 0.6) ^a	0.5 (0.3, 0.8) ^{ab}	0.5 (0.3, 1.3) ^b	0.035
Isobutyrate	0.6 (0.1, 1.2)	0.7 (0.1, 1.4)	0.6 (0.1, 1.2)	0.985	1 (0.6, 1.3) ^a	1.3 (0.8, 1.9) ^b	1.3 (0.8, 2.1) ^b	0.037
Isovalerate	0.7 (0.3, 1.6)	0.6 (0.1, 1.7)	0.8 (0.2, 1.8)	0.505	0.6 (0.3, 1.1)	0.9 (0.5, 1.3)	0.8 (0.4, 1.5)	0.196
Lactate	3 (1.1, 12.8)	2.9 (0.9, 11)	3.1 (1, 11.9)	0.902	0.2 (0.1, 0.7)	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)	0.536
Succinate	4.6 (2.1, 11.6) ^a	3.2 (1.1, 10.7) ^a	15.8 (2.9, 31.9) ^b	<0.001	0.7 (0.2, 3.3) ^a	5.7 (0.5, 24.4) ^b	5.2 (0.4, 25.6) ^b	0.017
Alanine	5.3 (2.5, 9.2) ^{ab}	4.7 (2.6, 7.8) ^a	5.6 (3, 10) ^b	0.079	1.9 (1.4, 2.8)	2.5 (1.7, 3.4)	2.5 (1.7, 3.5)	
Fumarate	0.1 (0.1, 0.4)	0.1 (0.1, 0.2)	0.1 (0.1, 0.3)	0.421	0.1 (0, 0.1)	0.1 (0.1, 0.1)	0.1 (0, 0.2)	0.220
Glucose	4.4 (1.6, 6.8) ^a	2.9 (1.5, 6.1) ^a	5.5 (2.5, 9.2) ^b	<0.001	3.6 (1.7, 8.3)	3.9 (2.3, 6.1)	3.5 (1.8, 5.8)	0.416
Isoleucine	1.2 (0.6, 2.4)	1.1 (0.5, 2.1)	1.3 (0.5, 2.3)	0.692	0.7 (0.5, 1.1)	0.9 (0.7, 1.3)	0.8 (0.5, 1.4)	0.397
Leucine	1.8 (0.9, 3.2)	1.6 (0.6, 3.2)	1.8 (0.7, 3.2)	0.769	1 (0.7, 1.5)	1.3 (1, 1.7)	1.2 (0.7, 1.7)	0.247
Malonate	1.8 (0.8, 4)	1.8 (0.7, 3.6)	1.6 (0.6, 3.9)	0.838	0.1 (0, 0.1)	0.1 (0.1, 0.2)	0.1 (0, 0.2)	0.539
Methanol	0.5 (0.2, 1)	0.6 (0.2, 1.2)	0.6 (0.2, 1.4)	0.707	0.4 (0.2, 0.8)	0.3 (0.2, 0.5)	0.3 (0.2, 0.5)	0.521
Phenylalanine	1.1 (0.4, 2)	1 (0.4, 2.5)	1.1 (0.5, 2.1)	0.982	0.5 (0.4, 0.9)	0.7 (0.5, 0.9)	0.7 (0.4, 0.9)	0.394
Tryptophan	0.2 (0.1, 0.2)	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)	0.539	0.1 (0, 0.1) ^a	0.1 (0.1, 0.2) ^b	0.1 (0.1, 0.2) ^{ab}	0.087
Tyrosine	0.7 (0.3, 1.5)	0.7 (0.3, 1.4)	0.8 (0.4, 1.9)	0.088	0.6 (0.3, 0.8)	0.7 (0.5, 1)	0.7 (0.4, 1)	0.257
Valine	1.8 (1.1, 3.3)	1.8 (0.9, 3.6)	2 (1, 3.8)	0.627	1.1 (0.7, 1.6)	1.2 (1, 1.9)	1.2 (0.8, 2)	0.446

^{a-d} Means within a row with different superscripts differ (Benjamini-Hochberg adjusted $P < 0.1$); * Kruskal-Wallis test

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Author names in bold designate shared co-first authorship.

What You Need to Know

Background and Context

Early-life microbiota has been linked to the development of food sensitization, but details of this association have not been fully elucidated.

New findings

Gut microbiota development in infancy with persistently low *Bacteroidetes* and high Enterobacteriaceae is associated with an increased risk of food sensitization, and a greater risk is observed among children born to Asian mothers or persistently colonized by *C. difficile*. Early-life microbiota maturation mediates the ethnicity-associated risks of food sensitivity.

Limitations

The use of 16S rRNA amplicon sequencing in this study may limit our understanding on functional capacities of microbiota, their low abundant members, and roles of other non-bacterial microbiota.

Impact

Our findings provide new insights into the role of early-life microbiota development in food sensitization that would support future development of microbiota-based interventions for preventing food allergy in toddlers.

Lay summary

Development of gut flora in the first year of life orchestrates risk of food allergy in children, and a higher risk was observed among Asian-Canadian children.