Infant Microbiota and Allergy Development in Early Childhood: A Systematic Review

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Abstract

Background: The prevalence of childhood allergic diseases has increased in recent decades, prompting investigations into contributing factors. Early life gut microbiome has become a key area of interest due to its potential influence on allergic disease risk.

Objective: This systematic review aims to synthesize current evidence on the relationship between early life gut microbiome composition and subsequent development of allergic diseases in childhood.

Methods: A comprehensive search was conducted in Medline, Science Direct, Scopus, and EMBASE databases. Longitudinal and repeated cross-sectional studies examining gut microbiome characteristics in infants less than one year of age and allergic outcomes in children older than 3 years were included. Two independent reviewers screened studies, extracted data, and assessed quality using the ROBINS-I tool.

Results: Twenty-six studies met the inclusion criteria. Consistent patterns emerged, including decreased microbial diversity and delayed microbiome maturation in infants who later developed allergic diseases. Taxonomic composition analysis revealed differential abundance of several bacterial taxa, with Bifidobacteriaceae frequently underrepresented and Enterobacteriaceae overrepresented in allergic infants.

Conclusions: This review provides evidence for an association between early life gut microbiome dysbiosis and increased risk of childhood allergic diseases. These findings highlight the potential for microbiome-based interventions in allergy prevention and the need for further research to establish causal relationships.

Introduction

Allergic diseases, including asthma, food allergies, and rhinitis, have become increasingly prevalent in recent decades, particularly among children in developed countries (Peters et al., 2017). This rise has prompted researchers to investigate potential environmental and biological factors that may contribute to the development of these conditions. One area of growing interest

is the role of the gut microbiome in early life and its influence on the development of allergic diseases.

The human gut microbiome, comprising trillions of microorganisms, plays a crucial role in shaping immune system development and influencing allergic disease risk (Savage et al., 2018). The first 1,000 days of life represent a critical window for microbiome establishment and immune system maturation (Éliás et al., 2023; Fontaine et al., 2023). Recent evidence suggests that the gut microbiota composition at 3-6 months of age may be particularly important in predicting later allergy risk (Sordillo et al., 2018).

Several environmental factors have been identified as potential modifiers of early gut microbiome composition. Mode of delivery (vaginal birth vs. Cesarean section) significantly impacts initial microbial colonization, with vaginal delivery exposing infants to a diverse array of maternal microbes and significantly increasing initial microbial colonization (Mitchell et al., 2020).

Feeding practices, particularly breastfeeding, provide essential nutrients and bioactive compounds that support the growth of beneficial bacteria (Lyons et al., 2020; Ames et al., 2023). Antibiotic exposure, especially in early life, can disrupt the developing microbiome and has been associated with increased allergy risk. Additionally, farm exposure and early presence of certain Lactobacilli species may have protective effects against allergy development (Johansson et al., 2011; Depner et. al., 2020).

Emerging research has highlighted associations between gut microbiome characteristics and allergic outcomes. Reduced microbial diversity and delayed microbiome maturation in infancy have been linked to an increased risk of allergic diseases. This decreased diversity may lead to impaired immune system development and a higher susceptibility to allergic sensitization. Furthermore, specific bacterial taxa, such as Lachnospira and Clostridium species, show altered abundance in infants who later develop allergies (Hoskinson et al., 2023). These alterations in microbial composition may influence immune responses through various

mechanisms, including the production of short-chain fatty acids and the modulation of regulatory T cells. Moreover, alterations in microbial composition associated with allergic diseases can lead to decreased production of short-chain fatty acids which, in turn, can alter T cell regulation which can contribute to allergic responses (Youn Yoo et al., 2020).

Recent studies have also explored the role of the airway microbiome in allergic diseases, revealing potential interactions between the gut and lung microbiomes in the development of asthma and allergies. This "gut-lung axis" has become an important area of investigation in understanding the complex etiology of allergic diseases. The cross-talk between these microbial communities may influence local and systemic immune responses, potentially contributing to the development or prevention of allergic conditions (Barcik et al., 2020; Chiu et al., 2020).

Despite these advances, significant research gaps remain. There is a need for longitudinal studies to establish causal links between early life gut microbiome composition and subsequent allergic outcomes. Such studies would help elucidate the temporal relationship between microbial changes and allergy development, as well as identify potential windows of opportunity for intervention. Additionally, the integration of multi-omics approaches, including metagenomics, metabolomics, and transcriptomics, could provide deeper functional insights into the mechanisms underlying these associations.

Given the potential for microbiome-based interventions targeting early life and the development of precision medicine strategies aimed at allergy risk reduction, a comprehensive synthesis of the current evidence is essential. Potential interventions may include targeted probiotic supplementation, prebiotic dietary modifications, or even fecal microbiota transplantation in high-risk individuals. However, the efficacy and safety of these approaches require thorough investigation before clinical implementation.

This systematic review aims to address the following research question: How does the early life gut microbiome influence later childhood asthma and allergies? By synthesizing

studies that have investigated the relationship between early life gut microbiome composition and subsequent allergic outcomes in children, we specifically aimed to:

1. Synthesize the current evidence on associations between specific gut microbiome characteristics in infancy and the development of allergic diseases in childhood.
2. Identify consistent patterns or discrepancies across studies regarding microbial diversity, specific taxa, or metabolic profiles associated with allergic outcomes.
3. Evaluate the quality and strength of the evidence, considering study designs, methodologies, and potential sources of bias.
4. Highlight key research gaps and future directions for investigation in this field.

Herein we provide a comprehensive overview of the current state of knowledge regarding the early life gut microbiome's influence on childhood allergies and asthma, informing future research efforts and potential interventional strategies. By consolidating and critically appraising the existing literature, we aim to contribute to the development of evidence-based approaches for allergy prevention and management, ultimately improving health outcomes for children at risk of allergic diseases.

Methods

We used the following search terms in Medline (OvidSP), and adapted them for Science Direct, Scopus, and EMBASE databases.

(("microbiota"[MeSH Terms] OR "microbiota"[All Fields] OR "microbiome"[All Fields])
OR ("metabolome"[MeSH Terms] OR "metabolome"[All Fields]))

AND (("Gut"[Journal] OR "gut"[All Fields])
OR ("feces"[MeSH Terms] OR "feces"[All Fields] OR "fecal"[All Fields])
OR ("intestines"[MeSH Terms] OR "intestines"[All Fields] OR] "intestinal"[All Fields])
OR gastrointestinal[All Fields])

AND ("infant" OR "toddler" OR "preschooler" OR "young child" OR "early childhood" OR "childhood"[MeSH Terms] OR “children”)

AND ("humans" OR "clinical population")

AND (asthma[MeSH Terms] OR "asthma"[All Fields] OR "wheezing" OR "bronchial hyperreactivity" OR "reactive airway disease" OR "allergy"[MeSH Terms] OR "allergy"[All Fields] OR "atopy" OR "hypersensitivity")

The authors used Covidence, a systematic review software, to screen studies and extract data. All team members participated in screening and data extraction. Abstracts were screened by one reviewer, then full texts were screened by two independent reviewers, and conflicts were resolved by a third reviewer. Data was extracted by two independent reviewers, and conflicts were resolved by consensus. Data extractors were paired randomly.

There were 6,887 studies imported from Scopus, EBSCOhost, and PubMed. Twenty-five duplicates were identified manually, and Covidence identified 2722 duplicates. In the title and abstract screening phase, 4,140 studies were screened, and 3,764 were identified as irrelevant. 376 studies were assessed for eligibility in the full-text screening phase. Three-hundred fifty studies were excluded, with the main reasons being the wrong study design, wrong follow-up time, and wrong patient population. In total, 26 studies were included for extraction.

We included studies that sequenced or analyzed the large-intestinal gut microbiota of infants one year of age or younger and which measured allergic disease outcomes at three years of age or older. Relevant allergic disease outcomes were defined as asthma, food allergies, non-food allergies, and/or food sensitivities. We excluded studies measuring only eczema or dermatitis, studies sequencing only the mother’s microbiome, studies of only the oral or airway microbiome, and studies whose only outcome was a measure of immune response other than IgE. We also excluded studies that were ongoing, literature reviews, and papers in a language other than English.

Extracted data included general information such as the study aim, study design, funding sources, and possible conflicts of interest. Information extracted on the interventions or exposures included a description of the intervention, a list of intervention groups, microbiome sequencing/analysis method, the 16S rRNA region, and fecal sample time points. Information on the outcome included outcome types, outcome measures, diagnostic criteria, and outcome time points. Compiled participant information included the population description, inclusion and exclusion criteria, larger birth cohort, and baseline population characteristics. We also extracted the methods of analysis and covariates adjusted for. Results extracted were the prevalence of outcomes, diversity patterns of the microbiota, taxonomic composition, specific metabolites and functional pathways, maturation patterns, additional relevant results, and a summary of conclusions.

To assess the quality of evidence, the authors used the *Risk of Bias in Non-Randomized Studies - of Interventions*, Version 2 (ROBINS-I V2) to evaluate the risk of bias in a specific result from non-randomized studies that examines the effect of an intervention on an outcome. Seven domains of bias are considered: risk of bias due to confounding, risk of bias in classification of interventions, risk of bias in selection of participants into the study (or into the analysis), risk of bias due to deviations from intended interventions, risk of bias due to missing data, risk of bias arising from measurement of the outcome, and risk of bias in selection of the reported result. There are four possible judgments of bias: low risk of bias, moderate risk of bias, serious risk of bias, and critical risk of bias. For each study, the two reviewers who extracted data also critiqued the quality of research using this tool.

Results

Out of 4,140 studies identified, 376 were included in full text screening, and 26 were included in data extraction. Twelve studies had incomplete data extraction at the time of writing, so the results reported in this review cover only 14 studies (Figure 1). Of the 14 studies, 10

looked at food allergy, 8 looked at asthma, 4 looked at rhinitis, and 5 looked at atopic sensitization. Ten of the studies used high-throughput sequencing (HTS) technology such as Illumina, microchip sequencing, or pyrosequencing and 4 used older or more targeted methods such as quantitative polymerase chain reaction (qPCR) or denaturing gradient gel electrophoresis (DGGE). Of the 10 using HTS, 7 directly compared characteristics of infant microbiota between disease groups, while 3 compared microbiota clusters associated with disease groups identified by unsupervised machine learning or supervised learning using baseline characteristics.

The following studies differ from the above-described groupings by providing some additional types of analysis. Hoskinson et. al., 2023 show that gut microbiome maturity score correlates to disease outcomes, and then analyze the characteristics that define maturity score. Tun et. al., 2021 and van Nimwegen et. al., 2011 provide mediation analyses with gut microbiome alterations as the mediator between baseline characteristics and disease outcomes. Tanaka et. al. 2017 use the results of HTS sequencing to pick specific taxa to analyze further using qPCR. Depner et. al., 2020 primarily make comparisons between clusters but additionally provide some direct comparisons by disease group. Kallio et. al., 2024, Chun et. al., 2023, Hoskinson et. al., 2023, Tun et. al., 2021, and Björkander et. al., 2020 include metabolite or multi-omics analyses, and Kallio et. al., 2024, Chun et. al., 2023, Hoskinson et. al., 2023, Stiemsma et. al., 2016, and Depner et. al., 2020 provide pathway analyses. Finally, Kallio et. al., 2024, Chun et. al., 2023, and Depner et. al., 2020 provide network analyses including bacterial taxa.

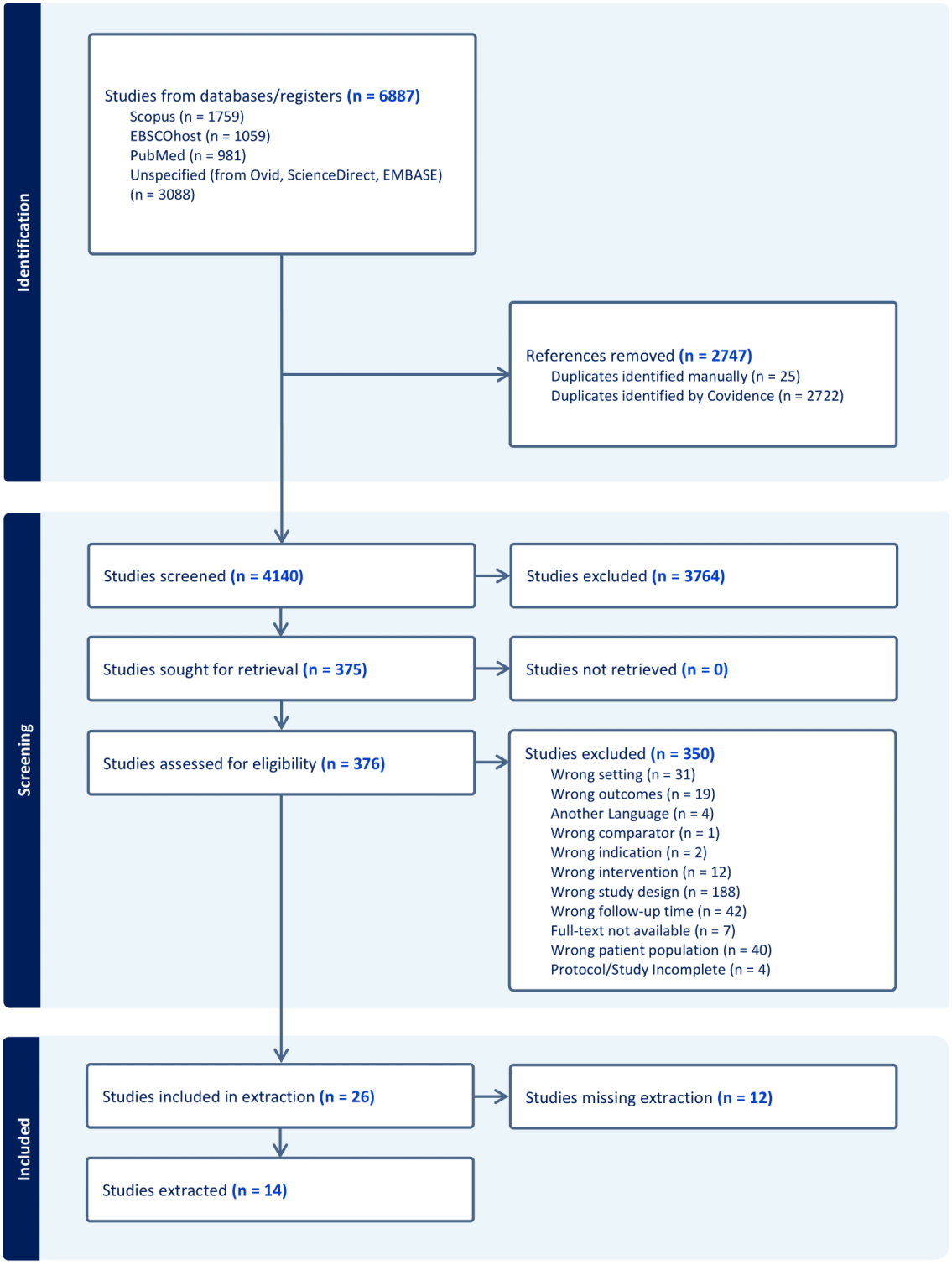

Figure 1. Flow Diagram of Records for Systematic Review

Map of all studies identified, screened, reviewed, and extracted with reasons for exclusion. Abstract screening was performed by one reviewer, full-text screening and extraction were performed by two independent reviewers.

Table 1: Summary of Included Studies

Publication	Country/ Sample Size/ Outcome Prevalence*	Sequencing	Diversity and Maturity**	Taxonomic Composition**	Functional Potential/ Metabolites**	Mediation/Causal Pathways
High-Throughput Sequencing—Disease Group Comparison						
Kallio et. al., 2024	**Country** Finland **Sample Size** Overall: 1,018 **Outcome Prevalence** Asthma: 15% Allergic rhinitis: 18% Food allergy: 8%	16S rRNA (V3–V4) via Illumina	α: no significant differences for any disease groups β: Bray-Curtis and generalized UniFrac at 3m significantly explain variance in PERMANOVA models predicting rhinitis Maturity: allergic rhinitis group showed delayed maturation characterized by ↓ bifidobacterium and slower acquisition of diversity during first 3m of life	Genus: ↓ Escherichia/Shigella for rhinitis; Genus: ↓ Bifidobacterium, ↓ Bacteroides for rhinitis with only one atopic parent; Genus: ↓ Bifidobacterium, ↓ Escherichia/Shigella, ↑ Actinomyces for rhinitis in association network	↓ pathways related to SCFA production, such as butyrate and propionate metabolism, ↓ metabolites associated with inflammation regulation for allergic rhinitis	Children with allergic rhinitis exhibited an altered gut microbiota composition even after adjusting for mode of delivery and breastfeeding duration.
Chun et. al., 2023	**Country** United States **Sample Size** Overall: 122 **Outcome Prevalence** Peanut allergy: 28.7%	16S rRNA (V3–V4) via Illumina	α: ↓ evenness for peanut allergy at infancy, increasing by mid childhood to match non peanut allergy	Species: ↓ Clostridium sensu stricto 1 sp., ↑ Bifidobacterium sp., ↑ Streptococcus sp. for peanut allergy	Differences in histidine metabolism pathway, differences in 139 metabolites incl. ↑ acetate, ↑ butyrate, ↑ propionate, ↑ isovalerate, ↑ isobutyrate, ↓ valerate for peanut allergy	Clostridium sensu stricto 1 sp. and butyrate were coexpressed in non-PA group but inversely expressed in PA group. Streptococcus sp. is related to histamine in Non-PA group, but not in PA group.
Hoskinson et. al., 2023	**Country** Canada **Sample Size** Overall: 1,115 **Outcome Prevalence**	Shotgun Metagenomic Sequencing	α: ↓ shannon diversity for any allergic diagnosis, asthma, and food allergy at 1y; Maturity: ↓ microbiota-derived age compared to	Species: ↓ Anaerostipes hadrus, ↓ Fusicatenibacter saccharivorans, ↓ Eubacterium hallii, ↓ Blautia wexlerae, ↑ Clostridium innocuum, ↑ Tyzzerella nexilis for any	Oxidative and Degradative Pathways: ↑ Sulfoquinovose degradation, ↑ D-Galactarate and D-Glucarate degradation, ↑ NAD(P)/NADPH	N/A

	Asthma: 165 Allergic rhinitis: 187 Food allergy: 136		chronological age for any allergic diagnosis, asthma, food allergy, and allergic rhinitis at 1y; Children with delayed microbiota maturation exhibited ↓ SCFA-producing species and ↑ potentially pathogenic species	allergic diagnosis	interconversion, Mucous Integrity Pathways: ↑ Cysteine disulfide bond reduction (implicated in mucous degradation), Fermentation Pathways: ↓ Methanogenesis from acetate, ↓ Secondary fermentation pathways, Protective Pathways: ↓ Sulfur oxidation, ↓ Fatty acid biosynthesis for allergy prone infants; Trace Amines (TAs): ↑ Phenylethylamine, ↑ Tryptamine, ↑ Tyramine SCFAs: ↓ Butyrate, Other Metabolites: ↑ D-Galactose, ↑ Trimethylamine, ↓ Nicotinic acid for allergy prone infants	
Joseph et. al., 2022	**Country** United States **Sample Size** Overall: 447 **Outcome Prevalence** Food allergy: 9.8% Egg allergy: 7.2% Peanut allergy: 5.8% Milk allergy: 2.0%	16S rRNA (V4) via Illumina	α: ↓ diversity for any IgE mediated food allergy, especially milk and peanut; Maturity: ↓ microbiota-for-age z-score (BMAZ) in IgE group at 1m and 6m	Species: ↓ Bacteroides sp., ↑ 2× Streptococcus sp., ↓ Lactococcus sp., ↓ Enterococcaceae sp., ↓ Erysipelotrichaceae sp., ↓ Varibaculum sp., ↑ Staphylococcus sp. for IgE at 1m; Order: ↑ Bifidobacteriales, ↓ Bacteroidales, ↓ Clostridiales, Species: ↓ 4× Lachnospiraceae sp., ↓ Roseburia sp., ↓ Faecalibacterium prausnitzii sp., ↓ Oscillospira sp., ↓ Ruminococcus sp., ↑ Peptoniphilus sp., ↓ Blautia sp.,	N/A	No mediating effect of eczema on relationship of alpha diversity, beta diversity, or BMAZ to food allergy

				↓ 7× Bacteroides sp., ↓ Bacteroides acidifaciens sp., ↑ Streptococcus sp., ↓ Carnobacterium sp., ↑ Enterococcus sp., ↑ 6× Bifidobacterium sp., ↓ 2× Enterobacteriaceae sp., ↓ Coprobacillus sp., ↑ Atopobium sp. for IgE at 6m		
Savage et. al., 2018	**Country** United States **Sample Size** Overall: 216 **Outcome Prevalence** Food allergy: 14/216 Food sensitization: 85/216	16S rRNA (V3–V5) via pyrosequencing	α: diversity and richness were similar between all groups	Genus: ↓ Haemophilus, ↓ Dialister, ↓ Dorea, ↓ Clostridium for food sensitization; Genus: ↓ Citrobacter, ↓ Oscillospira, ↓ Lactococcus, ↓ Dorea for food allergy; Clostridium association for food sensitization was reduced in magnitude and changed directions in African-American and solid food strata.	N/A	N/A
Tanaka et. al., 2017	**Country** Japan **Sample Size** Overall: 58 **Outcome Prevalence** Asthma: 16% Food allergy: 25% Other allergy (incl. asthma): 27%	16S rRNA (V1–V2) via Illumina; 16S rRNA (V6–V8) via pyrosequencing	α: no significant difference in diversity at 1m, 2m, or 6m; ↓ diversity by # of OTUs and PD whole tree at 1y for food allergy vs no allergy; Maturity: less complex, less adult-like microbial composition at 1y for food allergy	Genus: ↓ Leuconostoc, ↓ Weissella, ↓ Veillonella for food allergy vs. no allergy at 2m; Genus: ↓ Clostridium, ↓ Uncl. Enterobacteriaceae, Species: ↑ C. paraputrificum, ↑ C. tertium at 1y for food allergy vs. no allergy	N/A	N/A
Stiemsma et. al., 2016	**Country** Canada **Sample Size** Overall: 76 **Outcome Prevalence** Asthma: 13.6%	16S rRNA (V3) via Illumina	α: no difference in Shannon diversity between asthma and control	Phylum: ↓ Firmicutes, Genus: ↓ Lachnospira at 3m; Species: ↓ Clostridium neonatale, Family: ↑ Lachnospiraceae, Genus: ↑ Rothia at 1y	↑ pathways relevant to neonatal necrotizing enterocolitis	N/A

High-Throughput Sequencing—Cluster Comparison						
Lehtimäki et. al., 2021	**Country** Denmark **Sample Size** Overall: 686 **Outcome Prevalence** Asthma: 22.3% Allergic rhinitis: 6.8% Inhalant sensitization: 23.7% Food sensitization: 14.7%	16S rRNA (V4) via Illumina	N/A	Urbanized gut bacterial profile at 1m and 1y associated with increased risk of asthma at 6y, and urbanized gut bacterial profile at 1y with increased risk of any sensitization; Genus: ↑ Akkermansia, ↑ Phascolarctobacterium, ↑ Bacteroides, ↑ Atopobium, ↓ Gemella, ↓ Streptococcus, ↓ Moraxella, ↓ Negativicoccus, ↓ Dolosigranulum, ↓ Bifidobacterium, Family: ↓ Lachnospiraceae at 1m characteristic of urbanization; Genus: ↑ Campylobacter, ↑ Veillonella, ↑ Lachnoclostridium, ↑ Proteus, ↑ Escherichia/Shigella, ↑ Fusobacterium, ↓ Bacteroides, ↑ Tyzzerella (4), ↑ Eggerthella, ↓ Sutterella, ↓ Ruminiclostridium (5), ↓ Ruminococcaceae UCG002, ↓ Alistipes, ↓ Barnesiella, ↓ Dorea, ↓ Tyzzerella, ↓ Prevotella (9), ↓ Collinsella, Family: ↑ Enterobacteriaceae, Order: ↓ Rhodospirillales, Group: ↓ Ruminococcus torques at 1y characteristic of urbanization	N/A	N/A
Tun et. al., 2021	**Country** Canada **Sample Size**	16S rRNA (V3–V4) via Illumina; qPCR	C1-C1 cluster trajectory associated with atopic sensitization overall	C1-C1 cluster trajectory associated with atopic sensitization overall and food sensitization.	C1-C1 cluster trajectory associated with atopic sensitization overall and food sensitization.	C. difficile adds to C1-C1 trajectory risk

	Overall: 1,422 **Outcome Prevalence** Atopic sensitization: 12.8% Food sensitization: 5.8% Inhalant sensitization: 10.1%		and food sensitization. α: ↓ richness (Chao1, OTUs), ↓ PD whole tree for C1-C1 across whole community and within Bacteroidetes phylum; ↓ Shannon and Simpson diversity at 1y; Maturity: limited shifts towards a more adult-like composition for C1-C1 compared to other trajectories	Family: ↑ E/B ratio, Genus: ↑ Clostridium, ↑ Veillonella, ↑ Bifidobacterium, ↓ Bacteroides, Species: ↑ Uncl. Clostridiaceae, ↑ Uncl. Enterobacteriaceae for C1 across timepoints; Genus: ↓ Bacteroides, ↓ Parabacteroides, ↑ Clostridium, ↑ Veillonella, ↑ Bifidobacterium, Species: ↑ Uncl. Clostridiaceae, ↑ Uncl. Enterobacteriaceae for C1 at 3m; Genus: ↓ Bacteroides, ↑ Ruminococcus (2), ↑ Bifidobacterium, ↑ Veillonella, Species: ↑ Uncl. Lachnospiraceae, ↑ Uncl. Enterobacteriaceae for C1 at 1y	↓ sphingolipid metabolism pathway, ↓ 3 different glycosphingolipid biosynthesis-related pathways for all C1 trajectories vs. C2-C2; ↓ succinate for C1-C1 vs. C2-C2 at 3m; ↓ propionate, ↓ valerate, ↓ isobutyrate, ↑ acetate for C1-C1 vs. C2-C2 at 1y; reduction in SCFA producers in C1-C1 trajectory	
Depner et. al., 2020	**Country** Austria, Finland, Germany, Switzerland **Sample Size** Overall: 720 **Outcome Prevalence** Asthma: 8.50%	16S rRNA (V4) via Illumina	Maturity: ↓ estimated microbiome age (EMA) at 12m for asthma; D3 cluster grouped together samples with irregular maturation and had much higher prevalence of asthma than other clusters at 2m and 12m; EMA correlated strongly with α-diversity but was independent of the primary compositional differences according to PCA analysis	Genus: ↓ Roseburia, ↓ Coprococcus for asthma development	↓ butyrate production, ↓ relative abundance of the gene encoding butyryl–coenzyme A (CoA):acetate–CoA-transferase (butyrate metabolism) for asthma	Microbiome maturation partially mediated the protective effect of farm exposure against asthma
Older Methods						
Björkander et. al., 2020	**Country** Sweden **Sample Size**	qPCR	N/A	Genus: ↓ Lactobacillus for allergy	↑ Chemokines at 6m	N/A

	Overall: 194 **Outcome Prevalence** Asthma: 30.2%					
Bisgaard et. al., 2011	**Country** Denmark **Sample Size** Overall: 411 **Outcome Prevalence** Atopic sensitization: 910/? Asthma: 27/229 Allergic Rhinitis: 28/162	growth in different media; DGGE	α: ↓ averaged band richness for specific IgE, skin prick test, eosinophil count, and allergic rhinitis	Family: ↑ Staphylococcaceae for specific IgE	N/A	N/A
Johansson et. al., 2011	**Country** Sweden **Sample Size** Overall: 58 **Outcome Prevalence** IgE-mediated allergies: 34.5%	qPCR	N/A	Species: ↓ B. bifidum at 1w for allergy; Genus: ↓ Lactobacillus, Species: ↓ B. bifidum at 2w for allergy	N/A	N/A
van Nimwegen et. al., 2011	**Country** Netherlands **Sample Size** Overall: 2,733 **Outcome Prevalence** Astma: 6.90% Wheeze: 8.00% Food sensitization: 21.60% Inhalant sensitization: 28.90%	qPCR	N/A	Species: ↑ Clostridium difficile for wheeze and asthma	N/A	Effects of mode and place of delivery on atopic outcomes are mediated by C. difficile colonization

*The denominator of outcome prevalence percentages is the total sample size

**Unless otherwise specified, the comparison group for disease comparisons is healthy controls and for cluster comparisons is all other clusters.

Diversity and Maturity

Out of nine studies comparing alpha or beta diversity, seven found decreased diversity in at least one allergic group and two found no difference. All six studies mentioning maturation patterns reported delayed maturation in at least one allergic group (Table 1). Statistically significant diversity differences are sporadic across different disease groups and diversity metrics, but the direction of effect is consistent.

Taxonomic Composition

The two most common genera found across studies to be differentially represented between groups were Bifidobacterium and Clostridium, with 5 studies each reporting differences. Two studies found the Bifidobacterium genus to be underrepresented in allergic disease groups (Kallio et. al., 2024; Lehtimäki et. al., 2021), one found it to be overrepresented (Tun et. al. 2021), and two studies found specific species in the Bifidobacterium genus that were overrepresented (Joseph et. al., 2022; Chun et. al., 2023). Two studies found the genus Clostridium to be underrepresented in allergic disease groups (Savage et. al. 2018; Tanaka et. al., 2017), one found it to be overrepresented (Tun et al., 2021), one found Clostridium innocuum to be overrepresented (Hoskinson et. al., 2023), and one found that some species were over- and others underrepresented (Tanaka et. al. 2017). Few other genera were differentially abundant in more than two studies.

The taxa reported to be differentially expressed across studies were most often part of the bacterial families Clostridiales (8 studies), Lachnospirales (7), Enterobacterales (6), and Lactobacillales (6). The differences in Clostridiales were largely driven by Clotridiaceae, especially Clostridium and closely related species. The differences in Enterobacterales were mostly driven by Enterobacteriaceae of several species, in Lachnospirales by Lachnospiraceae, and in Lactobacillales by a range of orders and species (Figure 2). While some families were reported to be differentially abundant more often than others, the taxonomic composition results

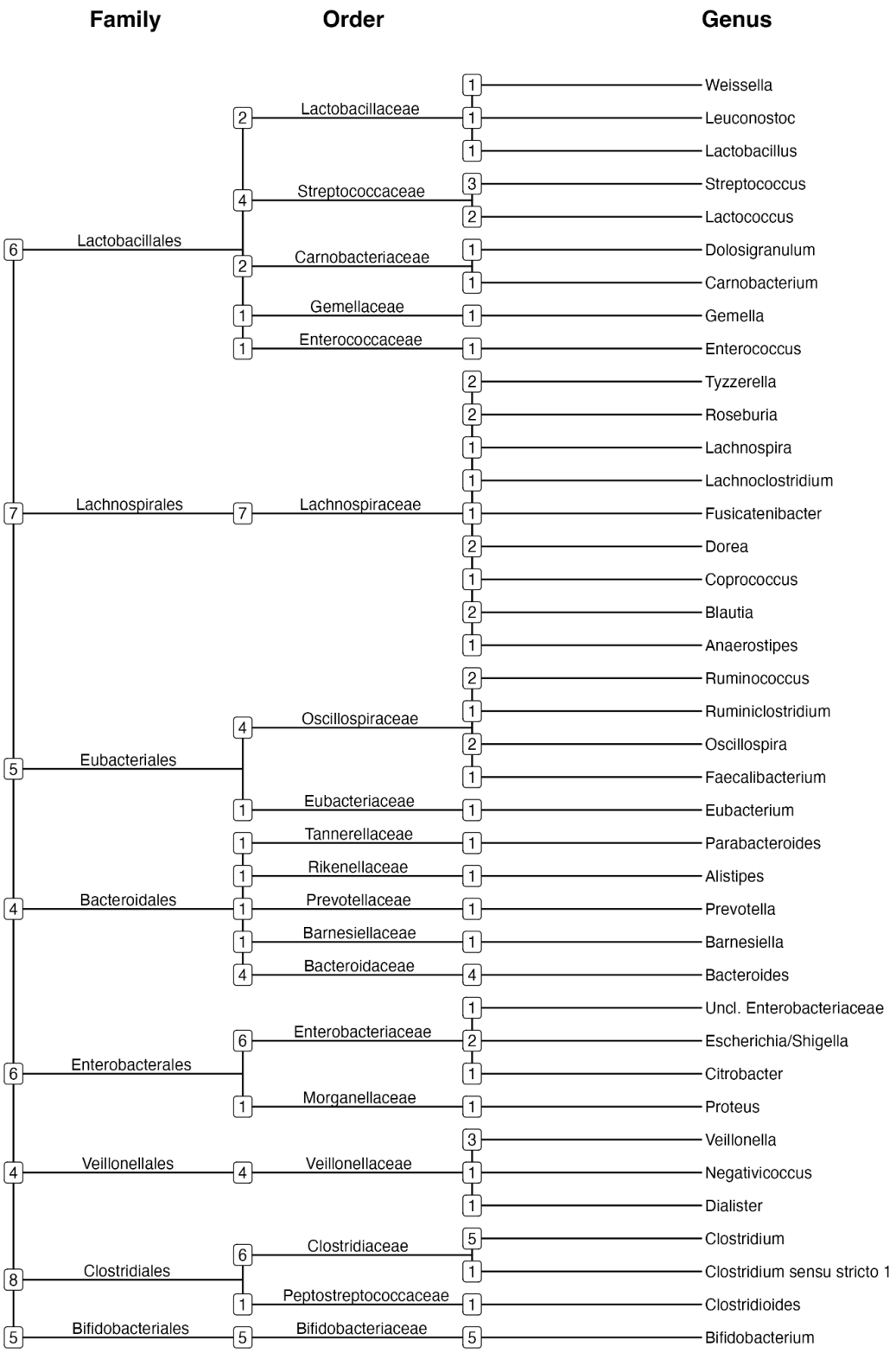

Figure 2. Summary of Taxonomic Composition Results

Tree diagram of differentially represented taxa in disease groups compared to controls or taxa characteristic of a cluster associated with disease in 14 extracted studies. Branches represent taxonomic relationships, and nodes are annotated with the number of studies reporting a given taxon or a descendant of it. Studies reporting multiple subtaxa within the same taxon (eg. multiple genera within the same order) are only counted once at each level. Studies reporting at the species level are summarized here by genus. One reported phylum and one reported group are not shown.

varied widely and the direction of association was inconsistent. For more details on the results of specific studies, see the annotated bibliography (Appendix B).

Pathways and Metabolites

Five studies report differential abundance of SCFAs or SCFA production pathways, especially butyrate and propionate. Some studies note differences in histidine metabolism, inflammation, and chemokines. Chun et. al., 2023 and Hoskinson et. al., 2023 each found many differentially expressed pathways and differentially abundant metabolites.

Mediation

Van Nimwegen et. al., 2011 and Depner et. al., 2020 found that gut microbiome composition was a mediator of the effects of mode and place of delivery and farm exposure respectively on allergic disease outcomes. Kallio et. al., 2024 found that gut microbiome composition was still correlated with allergic rhinitis after adjusting for mode of delivery and breastfeeding. Some data extraction is missing for the studies including network analyses.

Discussion

Our results clearly show that gut microbiome dysbiosis in infancy, including decreased microbial diversity, delayed maturation, and compositional shifts, are associated with various forms of allergic disease. While overall compositional changes are clearly associated with allergic disease, it's not clear that there are specific taxa driving these associations. Due to the large variability in the gut microbiome from person to person, it seems more likely to find shifts in certain functional areas than specific taxa. It's also not clear how much of a causal role the gut microbiota plays in development of allergic disease and which causal pathways are driving the association.

Many of the findings of the studies in this review support the hypothesis that overall gut dysbiosis, rather than specific taxa, is associated with allergic disease. The diversity and

maturity results are very consistent, while the taxonomic composition results vary widely—almost no species was found to be differentially abundant by more than one study (data not shown). However, the taxonomic composition results seem congruent with the maturity results, since the early-life microbiota typically shifts from being dominated by Enterobacteriaceae (overrepresented in allergic infants in some studies) in younger infants to Bifidobacteriaceae (underrepresented in allergic infants in some studies) in older infants (Fusco et. al., 2023). Enterobacteriaceae are also commonly observed with reduced diversity (Tanaka et. al., 2017), and Bifidobacteria "predominate in the gut microbiota of breastfed infants" (Kallio et. al., 2024).

The reviewed studies hypothesize that gut dysbiosis causes allergic disease by affecting the production of short-chain fatty acids (SCFAs), which regulate immune functions such as T cell and cytokine production, and by affecting the airway microbiome. This hypothesis is supported by many studies which found differential abundance of major SCFA producing bacteria such as Lactobacillaceae, Lachnospiraceae, and Clostridium, and several studies which found differential abundance of important SCFAs such as propionate and butyrate (Chun et. al., 2023; Hoskinson et. al., 2023; Tun et. al., 2021). Butyrate and butyrate-producing bacteria are mentioned particularly often.

While the above hypothesized pathway seems plausible and well-supported, there are many possible causal pathways that do not include the gut microbiome but are correlated with it. The gut microbiome may be a marker of overall health, and may contain more information about an individual's health than the included confounding variables. In fact, none of the studies reviewed included covariates intended to proxy for overall health, so this possibility is not well-addressed in the existing literature. Asthma, specifically, may be unrelated to the gut microbiome but highly correlated to the airway microbiome, which may be more directly related to asthma. However, at least one study included both gut and airway microbiome analyses and found that both were independently related to development of asthma (Lehtimäki et. al., 2021).

The hypothesized pathway is made more complex by a clear age interaction with the effect of gut dysbiosis on development of allergic disease. Tanaka et. al., 2017 showed that infants who later developed a peanut allergy had clear differences in the composition of their gut microbiome, but that the differences had stabilized by mid-childhood. Other studies showed that the effects of an immature gut microbiome became more significant when they persisted until one year of age (Lehtimäki et. al., 2021).

Whether or not the association presented in this review represents a causal relationship, the results could be useful for the purpose of early detection or preventative treatment. Tanaka et. al., 2017 were able to clearly separate food allergy, other allergy, and no allergy groups on a PCA plot whose axes were associated strongly with the presence of two genera of bacteria. A simple lab test based on a fecal sample could be developed to test for warning signs of later allergic disease. Kallio et. al., 2024 studied a cohort that was originally designed to test a probiotic intervention intended to prevent allergies. The probiotic treatment was effective both at altering the gut microbiome and at preventing some allergic diseases despite the fact that the effect on allergic disease did not seem to operate through changes in the gut microbiome. The treatment consisted primarily of bifidobacteria, the only species found to be differentially abundant in 5 out of 14 studies (and 9 out of the total 26). This indicates that probiotic treatments based on the taxa identified in this review might be effective.

The studies included in this review were largely rigorous and used advanced statistical methods, thus it would be difficult to perform an intensive assessment of the procedures, but we did note some limitations. Some studies did not correct for multiple testing on the basis of exploration (Johansson et. al., 2011) and cluster comparison studies typically did not look at statistically significant compositional differences between clusters, but rather looked at taxa “characteristic” of a certain cluster and performed statistical tests for differences in outcome between different clusters. The lack of statistical tests in some studies is both a limitation and a strength. While the results are less reliable, they provide more potential for discovery. The

results table and plots in this review included all significant taxonomic results, as reported, for studies that did statistical tests, regardless of whether the p-values were adjusted or not. For studies that did not do statistical tests of differential abundance, plots and tables include all reported results.

A few other limitations of the studies in this review include the following:

- There was not transparent reporting on use of covariates in all studies. Some studies reported controlling for confounding variables, but did not explain how they were included in the analyses.
- Studies that don't include any mediation analysis and control for many baseline covariates are controlling away the indirect effect of early life microbial exposures on allergic disease through the gut microbiome.
- Many studies did not report on subjects excluded due to missing fecal samples or missing outcome data. Only two studies analyzed the characteristics of the excluded subjects (Chun et. al., 2023; Joseph et. al., 2022).

This review also has some major limitations. Firstly, the reviewers were largely unfamiliar with the subject matter before beginning the review. Only one reviewer had experience with microbiome research, and none had experience with allergy research. Our inexperience led to some potentially biasing decisions made during the review. For instance, we decided to exclude studies of only eczema, without realizing that eczema is often a precursor to other allergic diseases and is considered an important risk factor. Because we are not experts and because the results are very complex, the extracted data are likely to be unreliable and incomplete. We provide here an overview of the main findings of the reviewed studies, but a more comprehensive data extraction and a meta-analysis would be necessary to fully understand the results.

We also note that we performed the risk of bias assessment using ROBINS-I, which was designed for interventional studies, and a more appropriate choice for our non-interventional

research question would have been the Risk Of Bias In Non-randomized Studies - of Exposure (ROBINS-E) tool.

Another severe limitation is that 12 out of 26 relevant studies were excluded due to missing data extraction. This review therefore includes only half of the studies that meet the original inclusion criteria. This limitation is mitigated by a preliminary look at the 12 excluded studies. The excluded studies had similar designs to the included ones (6 high-throughput sequencing (HTS) with disease comparison, 3 HTS with cluster comparison, 2 using older methods, and 1 using HTS but whose only relevant result was a mediation analysis) and the three most recent studies available were included (Kallio et. al., 2024; Chun et. al., 2023; Hoskinson et. al. 2023). Based on a preliminary look, the 12 excluded studies had similar taxonomic composition results to the 14 included (Appendix A, Supplementary Figure 1).

Conclusion

Herein, we synthesized evidence from 14 longitudinal studies which examine the associations between infant gut microbiome characteristics and subsequent development of allergic diseases in childhood. The reviewed studies demonstrated that gut microbiome dysbiosis in infancy, which is characterized by decreased microbial diversity, delayed maturation, and compositional shifts, was associated with an increased risk of various allergic outcomes including food allergy, asthma, and allergic rhinitis.

While overall compositional changes were clearly linked to allergic disease development, there was substantial heterogeneity in the specific taxa implicated across studies. The most commonly reported differentially abundant genera were Bifidobacterium and Clostridium, however, the direction of association varied. Several studies found decreased abundance of short-chain fatty acid (SCFA) producing bacteria and lower levels of SCFAs, particularly butyrate, in infants who later developed allergies.

The reviewed evidence suggests that gut microbiome dysbiosis may influence allergy risk through effects on immune development, potentially mediated by SCFA production and other metabolic pathways. Despite this, the observational nature of the included studies precludes definitive causal inference. Further research is needed to understand the causal mechanisms and evaluate the potential for microbiome-based strategies in allergy prevention.

Key limitations of this review were the exclusion of 12 relevant studies due to incomplete data extraction, potential for unmeasured confounding in the primary studies, as well as heterogeneity in methods of microbiome and outcome assessment. Future reviews would benefit from more comprehensive data extraction and quantitative synthesis of results where possible.

Despite these limitations, this review provides evidence of an association between early life gut microbiome characteristics and childhood allergy risk, demonstrating the potential importance of the infant gut microbiome in allergic disease development. These findings are important to future research efforts aimed at microbiome-based interventions for allergy prevention in high-risk infants.

Conflict of Interests

The authors declare no conflicts of interest.

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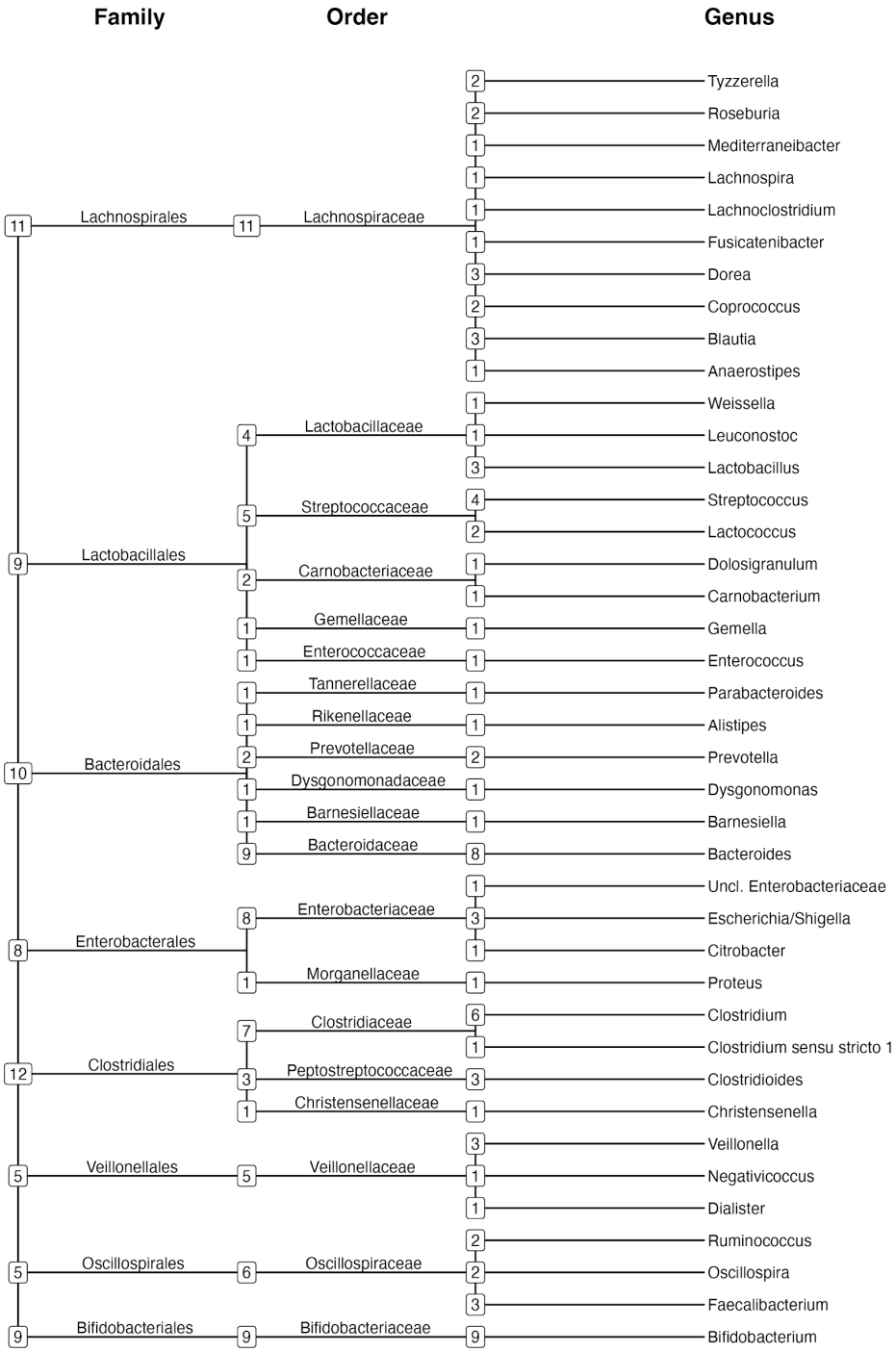
Appendix A: Supplementary Materials



Supplementary Figure 1. Summary of Taxonomic Composition Results—All Studies
Tree diagram of differentially represented taxa in disease groups compared to controls or taxa characteristic of a cluster associated with disease in all 26 relevant studies. Branches represent taxonomic relationships, and nodes are annotated with the number of studies reporting a given taxon or a descendant of it. Studies reporting multiple subtaxa within the same taxon (eg. multiple genera within the same order) are only counted once at each level. Studies reporting at the species level are summarized here by genus. One reported phylum and one reported group are not shown.

Appendix B: Annotated Bibliography

Kallio 2024

Title: Early-life gut microbiota associates with allergic rhinitis during 13-year follow-up in a Finnish probiotic intervention cohort.

Summary: Examined the association between the infant gut microbiota and allergic morbidity (asthma, allergic rhinitis, eczema, food allergy) in childhood until 13 years of age in a subgroup of the FLORA probiotic intervention cohort. One group received a probiotic mixture (four strains plus galacto-oligosaccharides) from the mother's 36th week of pregnancy to six months postpartum; the other, placebo. Fecal samples were collected at three months and were analyzed via 16S rRNA sequencing targeting the V3-V4 region. Allergic rhinitis was defined as recurrent symptoms (nasal discharge, blockage, sneezing) during allergen exposure, confirmed by IgE sensitization; asthma, by at least two physician-diagnosed wheezing episodes or persistent respiratory symptoms; food allergy, by positive oral food challenge; and eczema, by the UK Working Party's criteria. Allergic sensitization (IgE) was assessed at ages two, five, and 13 years via blood tests and skin prick tests (SPT) for common allergens (cat, dog, birch, timothy, mugwort, house dust mite, cow's milk, egg, wheat, peanut). Permutational multivariate ANOVA examined microbiota variation in relation to covariates that included birth mode, birth weight, breastfeeding duration, maternal atopy, infant antibiotic use, and socioeconomic status, using univariate and multivariate models. Differential abundance testing (adjusting for confounders) analyzed bacterial genera. Path models explored the association network between early-life factors, gut microbiota, and rhinitis. Wilcoxon and t-tests compared normally and non-normally distributed variables. Asthma prevalence was approximately 15% allergic rhinitis was approximately 18%, and food allergy was approximately 8%. Early-life factors significantly explained microbiota variation. Three-month gut microbiota was associated with birth mode, antibiotic use (0–6 months), exclusive breastfeeding, and probiotic treatment (probiotic treatment showing the strongest effect). No significant differences in bacterial genera relative abundance were found between allergy groups (any allergy, asthma, eczema, food allergy). However, Haemophilus and Escherichia/Shigella showed significant associations with atopic outcomes that persisted after adjusting for confounders. Children with allergic rhinitis exhibited delayed microbiota maturation. In conclusion, early gut microbiota composition and diversity were linked to allergic rhinitis, with lower bifidobacteria and slower maturation associated with increased risk of allergic outcomes, highlighting the potential of early-life gut microbiota as a target for allergy prevention.

Risk of Bias: Moderate risk of bias

Quality Assessment: The authors mention that only a subset of the original cohort had fecal samples available for microbiota analysis at 3 months. This availability could be related to factors that occurred after birth (e.g., breastfeeding difficulties, illness requiring medical attention) which might be linked to both the intervention and the outcome. Possibly. As mentioned above, factors influencing sample availability might be associated with probiotic use (e.g., parents more focused on gut health might be more diligent about providing samples).

It's possible that outcome measurement differed between intervention groups due to variations in: How parents interpret and report conditions like eczema and wheeze, potentially influenced

by their knowledge of the child's birth mode. How doctors diagnose asthma, which could be influenced by parental reporting of symptoms, which might be subject to the same biases mentioned earlier. Although less subjective, variations in blood collection or processing procedures between the groups could influence sensitization measurement.

Although the FLORA cohort originally had 872 fecal samples at 3 months, only 383 participants had available samples for this study due to previous investigations using samples, which could be linked to other health factors related to allergy development or intervention assignment (i.e.., maternal probiotic use) that would have affected the overall findings. Additionally, for the follow-up at 10 and 13 years, the authors relied on participant questionnaires for allergy status which is subject to recall bias. No sensitivity analyses or assessments for missingness were performed.

Chun 2023

Title: Longitudinal dynamics of the gut microbiome and metabolome in peanut allergy development.

Summary: None

Risk of Bias: Low risk of bias except for concerns about uncontrolled confounding

Quality Assessment: The characteristics of included subjects were similar to those of the excluded, so the exclusion is not likely to be related to the true outcome.

The authors conducted two sensitivity analyses in this study – one that adjusted for additional covariates including race/ethnicity, asthma, and number of allergen sensitizations and another comparing children who eat peanuts against children with a peanut sensitization without history of ingestion. The findings had no difference on the significance of the results. The characteristics of included subjects were similar to those of the excluded, so the exclusion is not likely to be related to the true outcome minimizing selection bias based on exclusion criteria. The sample size decreased greatly in mid-childhood, likely due to the nature of the study design (i.e., participants were only instructed to provide a sample at baseline but were invited to provide the follow-up sample). Because of this, there is little reason to suspect that missingness is related to the outcome and would interfere with the findings.

Hoskinson 2023

Title: Delayed gut microbiota maturation in the first year of life is a hallmark of pediatric allergic disease.

Summary: Investigated the dynamics of the gut microbiome and metabolome in relation to the development of peanut allergy (PA) through a longitudinal approach. The study focused on the composition of the gut microbiome and metabolite levels in infants who were at risk for PA but did not have the allergy at baseline. Researchers assessed changes in these profiles through mid-childhood in children who did and did not develop PA, exploring the relationship between temporal changes in gut microbiota and metabolite levels associated with the development of PA.

This was a case-control study that followed a birth cohort consisting of 1115 infants. Covariates adjusted for included child's sex, ethnicity, maternal and paternal atopy, presence of older siblings, mode of delivery, birth weight z-score, season of birth, breastfeeding status, antibiotic usage by age one, and exposure to environmental nitrogen dioxide (NO2). This study utilized multivariable conditional logistic regression to assess the odds ratios for developing atopic or allergic diagnoses, including food allergy, asthma, and allergic rhinitis, while controlling for early-life and familial exposures. A forest model predicted microbiota age, evaluated with a linear mixed-effect model adjusting for age and sample collection site. The Shannon diversity index measured microbiota diversity, while the MaALin package investigated associations between microbial community structures at 3 months and 1 year, adjusting for collection age and study center. Stool metabolites were analyzed using nuclear magnetic resonance (NMR) and liquid chromatography-tandem mass spectrometry (LC-MS/MS), with normalization and permutational multivariate analysis of variance (PERMANOVA) for microbiota maturation associations. Additionally, structural equation modeling (SEM) evaluated the direct and indirect effects of predicted microbiota age on allergic outcomes, corrected for multiple testing with the Benjamini–Hochberg method, highlighting significant associations with a false discovery rate (FDR) < 0.1. 165 participants developed asthma, 136 developed food allergy, and 187 developed allergic rhinitis. The study concluded that early-life influences and microbiome characteristics are consistently associated with four distinct allergic diagnoses at age five—specifically atopic dermatitis, asthma, food allergy, and allergic rhinitis. It suggested that impaired microbiota maturation at one year of age may universally relate to pediatric allergies, implicating compromised mucous integrity, elevated oxidative activity, decreased secondary fermentation, and heightened levels of trace amines as significant mediators in the relationship between microbiota maturation at one year and allergic diagnoses at age five.

Risk of Bias: Low risk of bias except for concerns about uncontrolled confounding

Quality Assessment: “Missing data were considered missing completely at random, and individuals were removed from the multivariable analysis if they had a missing value in any covariates”

This study had a large sample size (n=1115) with extensive clinical data available for the participants included in analysis. The authors considered any missing data to be missing completely at random and removed individuals with any missing covariate data from the multivariable analysis. They acknowledge that the investigators were not blinded during assessment which could have introduced observer bias. The risk of bias for this study overall is still low due to the objective measures of allergic disease, strong adherence to follow up, exclusion criteria not related to the outcomes of interest, and large sample size.

Joseph 2022

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

Summary: Aim: investigated the association between infant gut bacterial composition and food-related atopy at age 3–5 years

Prospective cohort study (WHEALS) that followed 447 infants. Fecal samples wereat ages one and six months. IgE-FA was determined retrospectively at ages 3–5 years using physician

panel review of clinical data and questionnaires collected from birth through age 3–5 years. The definition of IgE-FA included at least two of the following three criteria for each allergen (egg, milk, peanut): (1) specific IgE ≥0.35 IU/ml, (2) a positive skin prick test, or (3) parental report of symptoms suggestive of food allergy and a specific IgE level ≥0.10 IU/ml. Children meeting at least two of these criteria underwent a physician panel review for final classification as IgE-FA.

Analysis methods: "Characteristics of excluded subjects compared to included subjects using ANOVA for numeric covariates and chi-squared test for categorical. Characteristics of IgE and Non-IgE subjects compared using Kruskal-Wallis for numeric covariates and Fisher's exact for categorical.

Covariates considered: sex, race, household income, maternal education, mother's age at birth, mother's marital status, maternal atopy, maternal history of allergies or asthma, prenatal ETS exposure, prenatal indoor pets, delivery method, first born child, breastfeeding at 1m, solid food introduction <4m, eczema by 2y

For IgE vs. Non-IgE: Differences in alpha diversity examined using generalized estimating equations with Gaussian link including time interactions, followed by only main effects if interactions were non-significant. Compositional differences assessed using PERMANOVA and individual operational taxonomic unit (OTU) tests for OTUs detected in >25% of samples using zero-inflated negative binomial models with Benjamini-Hochberg adjusted p-values. Maturity differences assessed by microbiota-for-age z-scores using random forest models of age predicted by abundance of OTUs. Mediation analysis of eczema at 2y as mediator of all microbiota metrics on IgE status."

Prevalence

- with IgE-FA (≥1 of 3 allergens): 9.8%
- Egg IgE-FA: 73% of IgE-FA; 7.2% of all
- Peanut IgE-FA: 59% of IgE-FA; 5.8% of all
- Milk IgE-FA: 20% of IgE-FA; 2.0% of all

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

Risk of Bias: Moderate risk of bias

Quality Assessment: Participants included and excluded from the study varied on several covariates likely to affect the intervention and the outcome. The potential for bias is mitigated by the fact that the distribution of covariates did not seem to have an effect on the outcome. The magnitude of the difference in characteristics was not given.

For this study, infants were excluded if they had insufficient data available for IgE-FA classification. This could potentially be related to the outcome related to the level of concern about allergy development, especially since the authors relied on parental reports of infant

symptoms for IgE-FA classification. Additionally, while there were covariates adjusted for including maternal atopic history and breastfeeding practices, additional environmental and lifestyle characteristics could have confounded the results. These factors combined with a lack of sensitivity analyses results in this study having a moderate risk of bias.

Savage 2018

Title: A prospective microbiome-wide association study of food sensitization and food allergy in early childhood.

Summary: Savage 2018 investigated alterations in the gut microbiota of infants who later develop food sensitization. This prospective cohort study used existing data from an interventional trial (VDAART) that involved high-dose vitamin D supplementation during pregnancy. Fecal samples were collected from children of ages 3-6 months. Food sensitization (IgE-mediated) and allergy (physician-diagnosed and IgE-confirmed) were assessed at age three. Analyses controlled for race/ethnicity, sex, delivery method, and infant feeding type at the time of sampling (breast milk, formula, or solids). Chi-squared tests compared confounders across outcome groups (sensitized/allergic vs. controls). Alpha diversity (Chao1 and Shannon indices) at the genus level were compared using t-tests. Negative binomial modeling, with no rarefaction, analyzed genus-level compositional differences, and stratified analyses assessed the robustness of findings to potential confounders. 16S rRNA sequencing (V3-V5 region) characterized the gut microbiome. Food sensitization affected 39% (85/216) of the cohort, while food allergy affected 6% (14/216). The study found no association between overall gut microbial diversity and food sensitization or allergy. However, seven genera were significantly underrepresented in the food sensitization and/or allergy groups. These findings, along with analyses of potential confounders, suggest a potential causal role for the microbiome in food allergy development.

Risk of Bias: Moderate risk of bias

Quality Assessment: The authors did not perform a quantitative bias analysis. They acknowledged potential limitations due to unmeasured confounders, such as those related to maternal diet, pet ownership, and antibiotic use. They also recognized the potential for misclassification due to the resolution of food allergy in some children.

The study had missing data for some outcomes: Specific IgE data: Not all children in the VDAART study had IgE levels measured for all six foods. The authors state, "Not all children had IgE measured to all six foods." Food allergy diagnosis: Relied on parent-reported, doctor-diagnosed allergy, which can be subject to recall bias and may not capture all cases.

In this study there is potential confounding from unmeasured variables (e.g., maternal diet, pet ownership, and antibiotic use) as well as missing outcome data for some individuals related to specific IgE levels and food allergy diagnosis which were considered missing at random. Moreover, food allergy characterization relied on parental reports putting it at risk of recall bias and potential misclassification, even if caretakers were asked about previous physician diagnosis. Multiple sensitivity analyses were conducted based on race, solid food introduction,

and delivery mode. Most of the associations between bacterial species and food sensitization/allergy remained similar in magnitude and direction. This, however, was not the case with *Clostridium* and sensitization in the African American subgroup and the subgroup introduced to solid food. The nature of food sensitization data collection, missingness of certain confounding out outcome data, and some of the sensitivity analyses results puts the findings of this study at a moderate risk of bias.

Tanaka 2017

Title: Signatures in the gut microbiota of Japanese infants who developed food allergies in early childhood.

Summary: Tanaka 2017 evaluated the relationship between gut microbiota in the first year of life and the development of allergies during the first 3 years of life. This prospective cohort study followed 56 infants from birth to three years of age. Fecal samples were collected at 1, 2, 6, and 12 months. Food allergy diagnosis was determined at three years of age, using the ISAAC questionnaire. Multiple logistic regression models were used to adjust for potential confounders such as: sex, mode of delivery, breastfeeding duration, and antibiotic use within the first six months of life. 16S rRNA via pyrosequencing was conducted to analyze bacterial compositions, and qPCR to identify abundance of specific species/strains. Alpha diversity assessed from rarefied OTU table using observed species (the number of detected OTUs), phylogenetic diversity (PD) whole tree and the Shannon-Weiner index. Beta diversity assessed using pairwise-weighted Unifrac distances and partial least square discriminant analysis. Reads from V1-V2 were matched with reads from V7-V8 to identify species more abundant in one group than the other, then specific primers were used for qPCR of those species. The Mann-Whitney U-test compared alpha and beta diversity and relative/absolute abundances between groups. Spearman's rank correlation assessed pairwise genus co-occurrences, and Mantel-Haenszel procedures performed stratified analyses. Asthma prevalence was 16%; food allergy prevalence was 25%; and other allergies, including asthma, affected 27%. No significant differences in microbiome diversity were observed at one, two, or six months. However, at twelve months, food allergy subjects exhibited lower alpha diversity (observed OTUs and phylogenetic diversity). Food allergy subjects showed delayed microbiota maturation, maintaining a less complex, non-adult-like composition at twelve months. At two months (lactation period), the food allergy group showed underrepresentation of lactic acid bacteria, particularly *Leuconostoc* and *Weissella*. PLS-DA analysis revealed greater differences in microbiota composition at twelve months than at two months. Twelve-month alpha diversity remained significantly lower in the food allergy group. Three *Clostridium sensu stricto* species were overrepresented in the food allergy group at twelve months.

Risk of Bias: Low risk of bias except for concerns about uncontrolled confounding

Quality Assessment: “However, this study had limitations due to small sample size, which did not provide enough power for statistical analysis. Although there found no significant

confounding in our dataset, new onset of allergies must result from complex interplay of genetic and environmental risk factors."

There is overall a low risk of bias for this study, however the small sample size (n=56) is a limitation of this study. The authors state how this reduces the statistical power that is needed to assess the interplay of genetic and environmental risk factors for allergy development. Thirteen out of the original sixty-nine enrolled participants were excluded either due to a lack of fecal sample and/or loss to follow-up, but no sensitivity analyses were conducted to assess the potential impact of missing results. This exclusion is not suspected to be related to the outcomes of interest, so the overall risk of bias remains low except for concerns about uncontrolled confounding.

Stiemsma 2016

Title: Shifts in Lachnospira and Clostridium sp. in the 3-month stool microbiome are associated with preschool age asthma.

Summary: Stiemsma et al. (2016) examined the impact of early gut microbiota on asthma diagnosis in preschool-aged children. This nested case-control study analyzed fecal samples from 39 asthmatic children and 37 controls at age 3 and 12 months, using data from the Canadian Healthy Infant Longitudinal Development (CHILD) Study. The researchers adjusted for factors such as antibiotic exposure, sex, delivery mode, breastfeeding duration, and parental asthma history. Logistic regression was used to evaluate associations between clinical variables and asthma. The composition of the gut microbiome was analyzed using 16S rRNA gene sequencing, and quantitative PCR (qPCR) was employed to confirm the sequencing results and quantify specific bacterial taxa. Ratios of Lachnospira and C. neonatale were calculated to assess their combined effects, and quartile analysis was conducted to further examine these ratios and individual taxa.Bacterial dysbiosis was observed in the stool of children diagnosed with asthma by age four, characterized by a decrease in Lachnospira and an increase in C. neonatale in the fecal microbiota of these asthmatic children. The reduction of Lachnospira may serve as an early indicator of asthma in preschool-aged children, highlighting the first three months of life as a critical period when the gut microbiome significantly influences the developing immune system.

Risk of Bias: Moderate risk

Quality Assessment: Knowledge of the intervention (birth mode) could have influenced the assessment of the outcome, particularly for eczema and wheeze, which rely on parental reports.

In this study, missing data were imputed with the mode of each of the clinical variables. The authors do not report on the scale of this missingness, but they did confirm that the subsets of samples by outcome and timepoint were representative of the entire cohort. No sensitivity analyses were conducted, but this study could be susceptible to recall bias as allergic diagnoses relied on parental reports of symptoms potentially differential by parental history of atopy.

Lehtimäki 2021

Title: Urbanized microbiota in infants, immune constitution, and later risk of atopic diseases.

Summary: Investigated whether urbanization was associated with the microbiota composition in the infants' body and early immune function, and whether these contribute to the later risk of asthma and atopic traits. This prospective cohort study followed 700 children from birth to age six. Airway microbiota samples were collected at ages one week, one month, and three months. Gut microbiota samples were collected at one week, one month, and one year. Immune mediators (cytokines and chemokines) were measured in airway lining fluid at one month and in blood plasma at six months. Pediatricians diagnosed asthma, eczema, allergic rhinitis, and food/aeroallergen sensitization at age six. Urbanization was assessed using land cover data. Asthma was diagnosed using a quantitative symptom algorithm. allergic rhinitis was determined by sensitization status and parental reports of nasal congestion, sneezing, or rhinorrhea. Sensitization was determined by positive skin prick test (≥3 mm wheal) or specific IgE (≥0.35 kU/L). Logistic regression models, adjusted for pet ownership, daycare attendance, household income, maternal and paternal education, breastfeeding duration, and passive smoking, calculated odds ratios (ORs) for atopic outcomes in relation to urbanization level (urban vs. rural, and urbanization gradient). Microbiota data were analyzed using Phyloseq; alpha diversity (observed richness and Shannon diversity) was calculated; immune mediators were log-transformed and centered log-ratio scaled. Linear models analyzed immune mediator differences. Asthma prevalence was 22.3%, allergic rhinitis was 6.8%, aeroallergen sensitization was 23.7%; and food sensitization was 14.7%. The study concluded that an urbanized infant microbiota increased the risk of asthma, eczema, and allergic sensitization at age six, suggesting that urban environments may foster a disease-promoting microbiota composition in infants.

Risk of Bias: Low risk of bias except for concerns about uncontrolled confounding

Quality Assessment: Used both dichotomized urban/rural and a gradient.

Authors checked for moving.

Some missing, still overall big sample size.

This study presents a low risk of bias based on the objective measures of allergy diagnoses and the unbiased land cover data to classify living environments. Participants were excluded from analysis due to missing address data and/or missing microbiota samples at follow-up timepoints. There was no sensitivity analysis done to assess the impact of missing data, and the authors acknowledge gaps in their data that has been previously associated with asthma and living environment (e.g., air pollution, exposure to nonbacterial items, maternal behaviors). Still, this study in itself has an overall low risk of bias except for concerns about uncontrolled confounding.

Tun 2021

Title: Ethnicity Associations With Food Sensitization Are Mediated by Gut Microbiota Development in the First Year of Life.

Summary: Tun et al. (2021) investigated the relationship between gut microbiota development in the first year of life and the development of food sensitization in children, focusing on the role of ethnicity and early-life exposures. This cohort study analyzed 2844 fecal samples from 1422 infants in the Canadian Healthy Infant Longitudinal Development (CHILD) Study that were collected at 3 and 12 months of age. Microbial composition was determined using 16S rRNA amplicon sequencing, and food sensitization was determined with skin prick tests at ages 1 and 3. Data analysis involved partitioning around medoids (PAM) clustering to identify microbiota trajectories and logistic regression to assess associations between trajectories and sensitization. Covariates adjusted for included delivery mode, feeding status, antibiotic use, sibling and pet presence, and maternal ethnicity and allergy. Prevalence of Atopic sensitization was 12% at age 1 and 12.8% at age 3, food sensitization was 9.5% and 5.8%, and inhalant sensitization was 3.3% and 10.1%, respectively. Microbiota diversity increased from 3 to 12 months, with greater abundance of Bacteroidetes and Firmicutes and reduced Proteobacteria and Actinobacteria at 12 months. PAM clustering identified four microbiota trajectories; the C1-C1 trajectory, characterized by persistently low Bacteroides and a high Enterobacteriaceae/ Bacteroidaceae ratio, was significantly associated with increased food sensitization at age 3 (adjusted OR 2.34, 95% CI 1.20–4.56) and peanut sensitization (adjusted OR 2.82, 95% CI 1.13–7.01). This association was stronger in Asian infants. Late-infancy C. difficile colonization further increased this risk. The C1-C1 trajectory was also associated with deficient sphingolipid metabolism. In summary, the study suggests that distinct early-life gut microbiota trajectories, influenced by ethnicity and other factors, are associated with increased risk of food sensitization, highlighting the importance of early-life gut microbiota development in allergy development. Limitations include the use of 16S rRNA sequencing (limiting functional insights), the observational nature of the study (precluding causal inference), and potential limitations in generalizability due to the study cohort.

Risk of Bias: Low risk of bias except for concerns about uncontrolled confounding

Quality Assessment: No bias analysis of excluded samples due to missing timepoint.

Questionnaire data in this study was only used for potential confounders and not outcome symptomatology, making recall bias not of major concern. This study had minimal missing microbiome data (<10% in early infancy and <1% in late infancy) and a large sample size overall, and the authors conducted sensitivity analyses that made the findings more robust. These analyses integrated microbiota and genetic factors to assess associations with food sensitization showing potential for predisposition based on ethnicity.

Depner 2020

Title: Maturation of the gut microbiome during the first year of life contributes to the protective

farm effect on childhood asthma.

Summary: Depner 2020 investigated the mechanisms by which growing up on a farm protects against childhood asthma, focusing on the role of gut microbiome maturation during the first year of life.

This prospective birth cohort study followed children in rural areas from birth until the age of six. Fecal samples were collected at 2 and 12 months of age for microbiome analysis. Asthma

diagnosis was determined at age six. Approximately half of the children are born to mothers living on farms. Covariates adjusted for included Mode of birth (vaginal vs. Cesarean), breastfeeding duration, antibiotic use, number of older siblings, pet exposure, maternal smoking during pregnancy, farm exposures (visits to animal sheds, consumption of farm milk/eggs), and dietary diversity. The study utilized multivariate logistic regression to assess the association between gut microbiome maturation (measured as Estimated Microbiome Age or EMA) and asthma risk. Random forest modeling was used to predict EMA and evaluate microbial community structures at 2 and 12 months of age. Mediation analysis was conducted to explore the extent to which EMA influenced the protective farm effect on asthma. Linear mixed-effects models were employed with study center as a random effect to account for variations between study locations. Asthma prevalence was 8.5%. Farm-exposed children exhibited higher alpha and beta diversity at both two and twelve months, along with accelerated microbiome maturation (higher EMA). Mediation analysis revealed that microbiome maturation partially mediated the protective farm effect against asthma. In conclusion, farm environments promoted gut microbiome diversity and accelerated maturation, enriching beneficial taxa and metabolites, and thereby reducing childhood asthma risk, highlighting the importance of early-life environmental exposures on health.

Risk of Bias: Low risk of bias except for concerns about uncontrolled confounding

Quality Assessment: Complete case analysis.

“For a sensitivity analysis, we defined ‘asthma after 3 years’ as an asthma diagnosis established in the fourth, fifth or sixth year of life. Wheeze phenotypes were derived from a latent class analysis as described previously”

“In a sensitivity analysis, we explored an extended list of 15 food items (main food items with the addition of eggs, fish, nuts, soy, margarine, chocolate, other milk products, cow’s milk and butter), which were dichotomized into at least 11 items. Furthermore, the children’s diet was assessed with respect to the kind of supplemental food and its introduction in terms of at least weekly consumption56. Farm milk consumption was defined as the weekly consumption of any milk obtained directly from a farm, irrespective of boiling or skimming”

“We clustered the samples over both time points and, as a sensitivity analysis, separately for both time points”

“To confirm that results were independent of the training sets, we performed sensitivity analyses by restricting the models to children who were not included in model building.”

“In addition, PCoA was also applied in a sensitivity analysis combining all samples from both time points.”

This study utilized a complete case analysis, excluding participants with missing sample data. However, the authors were very thorough conducting several sensitivity analyses to ensure the validity of their findings. This includes analyses that defined an asthma diagnosis after 3 years, an extended list of fifteen food items for sensitization, timepoints separately and combined, restricting data that was not used in random forest regression model building, and incorporating a principal-coordinate analysis with all combined samples. Parent reports of asthma were used, but lung function measurements validated outcome definition. The findings of this study have a low risk of bias except for concerns about uncontrolled confounding.

Björkander 2020

Title: Childhood allergy is preceded by an absence of gut lactobacilli species and higher levels of atopy-related plasma chemokines.

Summary: Investigated the association between gut lactobacilli and Th-related plasma factors in allergy development during childhood. This prospective cohort study followed 194 children from birth until 10 years of age. Fecal samples were collected from 65 children between one week and two months of age to assess the presence of *Lactobacillus casei*, *L. paracasei*, and *L. rhamnosus*. Plasma samples were collected at six months and ages 1, 2, 5, and 10 years to measure chemokines and cytokines. Lung function (FeNO and spirometry) was also assessed at age 10. Allergy status was determined at one year of age and subsequently at 2, 5, and 10 years. Allergy was defined at age one and subsequently at ages 2, 5, and 10, using skin prick tests (SPT; positive if wheal diameter ≥3 mm after 15 min) and allergen-specific IgE (positive if level ≥0.35 kU/L); allergy was diagnosed with a positive SPT or IgE and at least one allergic symptom (eczema, food allergy, asthma, rhinoconjunctivitis). Non-parametric tests (Chi-squared, Fisher's exact, Mann-Whitney U-test) were used for comparisons. Logistic regression, adjusting for parental allergy, modeled chemokine-allergy associations. Analysis used GraphPad Prism 7. Allergy prevalence at 10 years was 30.2%. The presence of lactobacilli in the first two months of life was associated with lower allergy prevalence at ages 1, 2, 5, and 10. Children with more frequent detection (3–4 occasions) were least likely to be allergic, particularly at ages one and two. This association was stronger among consistently allergic/non-allergic children and persisted even in those with a family history of allergies, despite lower lactobacilli prevalence in this group. Gut microbial diversity increased significantly from one to twelve months. *Enterobacteriaceae*, *Enterococcaceae*, yeast, and fungi increased in abundance; *Staphylococcaceae* decreased. High *Staphylococcaceae* at one month was associated with allergic sensitization, though not significant at twelve months. This suggests a potential role for reduced *Staphylococcaceae* in allergy development. In summary, infant gut lactobacilli were associated with reduced allergy risk and more favorable immune profiles, potentially contributing to allergy protection.

Risk of Bias: Serious risk of bias

Quality Assessment: Authors did not perform a quantitative bias analysis or use negative controls. They acknowledged the limitations of their study in identifying all potential confounding factors. They mentioned that other factors, such as the infant's age at the time of fecal collection, the age of allergy diagnosis, and whether the lactobacilli were investigated at the genus or species level, could impact the results.

NO. The authors did not employ any specific alternative methods to correct for the potential bias introduced by missing data. They mainly relied on the complete case analysis. NO. There is no clear evidence presented to demonstrate that the results were not biased by missing data. The authors did not explicitly discuss the potential impact of missing data on their findings or conduct sensitivity analyses to explore this.

The authors do not include any covariates in their analysis but acknowledge potential confounding factors including infant age at the time of fecal collection. They did not list other common confounders like antibiotic use and pet exposure which they did not adjust for either. Additionally, only 65 out of the cohort of 281 infants had available samples which greatly

reduces the power and generalizability of the findings. This was conducted as a complete case analysis, and the authors did not explicitly discuss the potential impact of missing data on their findings or conduct sensitivity analyses to explore this.

Bisgaard 2011

Title: Reduced diversity of the intestinal microbiota during infancy is associated with increased risk of allergic disease at school age.

Summary: Bisgaard et al. (2011) investigated the relationship between early infant gut microbiota diversity and the development of atopic disorders by age six. This cohort study used the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) data and followed 411 high-risk infants, born to mothers with asthma, for six years. Fecal samples were collected at age 1 and 12 months. Bacterial diversity was assessed using 16S rRNA PCR with denaturing gradient gel electrophoresis (DGGE) to determine band richness, and conventional culturing to identify specific species. Researchers took into consideration confounders including mode of delivery, parental smoking, infant feeding, antibiotic use, birth period, number of siblings, daycare attendance, and pet ownership. Logistic regression was used to examine the relationship between bacterial groups, band richness, asthma, and allergic rhinitis. Generalized Estimating Equations (GEE) assessed specific IgE levels, skin prick test results, and peripheral blood eosinophil counts at four time points, with associations between IgE and skin prick test results analyzed using a log-linear model and eosinophil counts evaluated through a continuous linear model. Cox regression analysis tested the differences in risk related to band richness and bacterial cultures for atopic dermatitis. Principal Component Analysis (PCA) was applied to analyze band classes and identify patterns in the data. The Pearson Correlation Coefficient calculated the correlation of band richness at 1 and 12 months, while PCA was also used to analyze "band richness" for bacterial diversity in each DGGE profile. Prevalence of asthma and allergic rhinitis at 6 years of age or earlier was 22.3% and 6.8% respectively. Prevalence of allergic, food, and aeroallergen sensitization was 31.7%, 23.7%, and 14.7% respectively. The study discovered that lower bacterial diversity in infants' gut flora raises the risk of allergic sensitization, allergic rhinitis, and high levels of eosinophils in the blood, but it was not linked to asthma. This indicates that an imbalance in the gut microbiome may play a role in the development of allergic diseases.

Risk of Bias: Moderate risk of bias

Quality Assessment: The authors did not perform a quantitative bias analysis. They acknowledged the limitations of their study in identifying all bacterial species in the gut flora due to the limitations of cultures and the potential loss of anaerobic bacteria during transportation. They also mentioned the potential for misclassification of atopic dermatitis based on clinical criteria. While these limitations suggest potential confounding, the authors argued that the prospective design, comprehensive microbiological characterization, and long-term follow-up with objective assessments are strengths of their study.

Only corrected for mothers with asthma

The study had missing data for some outcomes: Specific IgE and skin prick test data were not reported for all assessments. Peripheral blood eosinophil counts were not reported for all assessments. Allergic rhinitis data was only available for 192 children at age 7. Asthma data

was only available for 282 children at age 6. The study had some missing data on confounding variables, particularly for those related to sociodemographics and environmental exposures.

Missingness and threats to external validity from cohort design

This study had missing data for multiple outcomes and potential confounders. This includes allergy data based on parental interviews, which is already a potential source of recall bias, and for the biological measures of allergy including specific IgE levels, skin prick test results, and peripheral blood eosinophil counts. Missing data were treated as missing observations meaning complete case analyses were performed, which is problematic as missingness was not consistent across outcome and no sensitivity analyses were performed. Additionally, the authors describe the potential for misclassification of atopic dermatitis based on clinical criteria and limitations in bacterial species identification due to culturing and sample transportation. There is also poor external validity as this study only looked at children of mother with a history of asthma, so generalizability of these findings is even further limited.

Johansson 2011

Title: Early colonization with a group of Lactobacilli decreases the risk for allergy at five years of age despite allergic heredity.

Summary: Johansson et al. (2011) investigated the influence of early gut microbiota on the development of IgE-mediated allergic disease by age five, considering parental allergy status. This cohort study followed 58 infants (35 with allergic parents, 23 with non-allergic parents) from birth to age five. Fecal samples were collected at 1 week, 2 weeks, 1 month, 2 months, and 12 months to quantify Bifidobacterium adolescentis, Bifidobacterium breve, Bifidobacterium bifidum, Clostridium difficile, a group of *Lactobacilli (Lactobacillus casei, Lactobacillus paracasei, and Lactobacillus rhamnosus), and Staphylococcus aureus by qPCR. The outcome of interest was determined at five years. Allergy was defined by a positive skin prick test and/or elevated allergen-specific IgE with symptoms. Covariates including mode of delivery, parental smoking, infant feeding, antibiotic use, birth period, number of siblings, daycare attendance, pet ownership, and were considered. Fisher's exact test was used to evaluate differences in frequencies between the groups of infants with allergic parents versus non-allergic parents, and also between children who developed allergy as compared to the ones remaining non-allergic. To assess differences in the relative amounts of the detectable bacteria the Mann-Whitney Rank Sum test was applied. Additionally, to investigate differences in the number of occasions with the bacteria the first two months, Mann Whitney Rank Sum test was used.Prevalence of IgE-mediated Allergies was 34.5%. The results indicate that parental allergies influence the gut microbiota of infants. Early colonization by specific Lactobacilli (L. casei, L. paracasei, L. rhamnosus) appears to reduce the risk of developing allergies by the age of five, even in those with a family history of allergies. Findings suggest that naturally acquired gut microbiota play a role in allergic diseases.

Risk of Bias: Moderate risk of bias

Quality Assessment: Missing data for one individual with small sample size n=58 and with subgroups

No correction for multiple testing was done, potentially generating false significances.

This study did not rely on subjective measures for allergy classification which strengthens its findings. It, however, had a small sample size (n=58) with demographic data missing from one individual and reduced power with the sub-grouping by parental allergy and IgE-mediated allergic disease at age five. The authors also acknowledge the potential for false significances from multiple testing with no correction. For these reasons, this study has a moderate risk of bias overall.

van Nimwegen 2011

Title: Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy.

Summary: VanNimwegen 2011 investigated the relationship between microbiota composition, mode and place of delivery, and atopic manifestations. This prospective cohort study followed children from birth to age 6-7 years. Fecal samples were collected at one month to assess gut microbiome composition. Asthma and food allergy were determined through physician diagnosis and IgE blood test. Logistic regression models, adjusting for recruitment group, sex, birth weight, breastfeeding duration, maternal smoking, maternal age, maternal education level, and age at fecal sampling, calculated unadjusted and adjusted odds ratios (95% CI) for asthma at age 6–7 years. Generalized estimating equations (GEE) analyzed repeated measures of eczema, wheeze, and sensitization to food and inhalant allergens. Mediation analysis, using the ab product coefficient method, evaluated whether *C. difficile* colonization mediated the association between delivery method and atopic outcomes. Analyses were limited to participants with complete data on delivery method, *C. difficile* colonization, and the specific atopic outcome. Asthma prevalence was 6.9%; food-allergen sensitization, 21.6%; and inhalant-allergen sensitization, 28.9%. The study concluded that: (1) infant gut microbiota composition is associated with early atopic manifestations; (2) both gut microbiota composition and delivery method are associated with atopic manifestations, especially in children with a family history of atopy; and (3) the mediating role of *C. difficile* strengthens the evidence for a causal pathway between delivery method and atopic manifestations.

Risk of Bias: Low risk of bias except for concerns about uncontrolled confounding

Quality Assessment: No sensitivity analyses were conducted in this study. The authors did perform unstratified and stratified analyses by parental atopy, and included several variables that could have potentially confounded findings. Only 4% of the initial population enrolled (n=2834) were excluded, so any risk of selection bias due to missing results should have minimal effect on the overall findings.