

Review

Predictors and biomarkers of food allergy and sensitization in early childhood



Erin C. Davis, PhD^{*}; Courtney M. Jackson, PhD^{*}; Tiffany Ting, BS^{*}; Albana Harizaj, MD^{*,†};
Kirsi M. Järvinen, MD, PhD^{*,†,‡}

^{*} Division of Allergy and Immunology, Center for Food Allergy, Department of Pediatrics, University of Rochester School of Medicine and Dentistry, Golisano Children's Hospital, Rochester, New York

[†] Division of Allergy, Immunology, and Rheumatology, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York

[‡] Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, New York

Key Messages

- Genetics certainly play a role in food allergy (FA) development; however, it is likely that interactions between risk alleles and environmental factors are responsible for such rapid increases in FA prevalence.
- Infants can be exposed to antigens through multiple routes in early life, perhaps initiating tolerance or sensitization to a food before complementary feeding. More comprehensive studies assessing maternal diet and antigen exposure throughout pregnancy and infancy are needed to inform dietary guidelines for high-risk families.
- Lifestyles that offer consistent and diverse microbial exposure through pet exposure, large families, and low use of antibiotics are protective against FA and atopic disease.
- The gut microbiome in infancy has been associated with later development of FA and food sensitization. Although microbial metabolites such as butyrate may be protective against atopic disease, a lack of studies employing metagenomic analysis techniques have limited our ability to determine microbial functional pathways that may be related to FA susceptibility.
- Increased production of proinflammatory and Th2 cytokines, altered immunoglobulin G and immunoglobulin A responses, and loss of regulatory T cells have been associated with FA; however, more studies are needed to investigate development of longitudinal specific antibody responses and innate and adaptive immune responses between infants with and without FA.
- More prospective birth cohort studies are needed to comprehensively evaluate how previously identified genetic and environmental risk factors in early life interact to influence microbial and immune trajectories and risk for FA in early childhood and identify biomarkers of early development or protection against allergic disease.

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ABSTRACT

Objective: To review existing literature on the early risk factors for and biomarkers of food allergy (FA) and food sensitization (FS) and highlight opportunities for future research that will further the understanding of FA pathogenesis in infancy and toddlerhood.

Data sources: PubMed search of English-language articles related to FA and atopic disease.

Study selections: Human studies with outcomes related to FA, FS, and other atopic disease in childhood were selected and reviewed. Studies published after 2015 were prioritized.

Reprints: Kirsi M. Järvinen, MD, PhD, Division of Pediatric Allergy and Immunology, University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave, Box 777, Rochester, NY 14642. E-mail: kirsi_jarvinen-seppo@URMC.Rochester.edu.

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Results: The prevalence of FA has greatly increased in recent decades and is now a global public health concern. A complex network of early life risk factors has been associated with development of FA and FS in childhood. Food allergy has a genetic component, but recent evidence suggests that interactions between risk alleles and other environmental exposures are important for disease pathogenesis, potentially through epigenetic mechanisms. Lifestyle factors, such as delivery mode, antibiotic use, and pet exposure also influence FA risk, which may be through their effect on the early life gut microbiome. How these early life risk factors, along with route and timing of antigen exposure, collectively target the developing immune system remains an ongoing and important area of study.

Conclusion: The current body of evidence emphasizes the first 1000 days of life as a critical period for FA development. More observational studies and adequately powered clinical trials spanning early pregnancy through childhood are needed to identify novel biomarkers and risk factors that can predict susceptibility toward or protection against FA.

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Introduction

It is estimated that 7.6% of the pediatric population in the United States suffers from food allergy, which is equivalent to about 1 in 13 children.^{1,2} Food allergy results from an abnormal response of the immune system to food antigens.³ Food allergy (FA) can be life-threatening and impact quality of life and represents a substantial economic burden for families.¹ Although sometimes outgrown, FA is often chronic and is also an independent risk factor for asthma and allergic rhinitis, which tend to develop later in childhood and adulthood.⁴

The prevalence of FA has notably increased over the last 30 years, with some estimating a 1.2% increase in prevalence per decade between 1988 and 2011.⁵ There is certainly a hereditary component to FA; however, such dramatic rises in FA rates, particularly in developed countries, are hypothesized to be a result of shifts in environmental exposures associated with a Westernized lifestyle. Onset of FA symptoms often occurs during infancy or toddlerhood and can begin as early as the first few months of life. Although groundbreaking clinical trials have shown that early introduction of allergenic foods is protective against FA development in high-risk groups,⁶ infants can be sensitized to allergens before the introduction of complementary foods, emphasizing the need for earlier interventions. Numerous lifestyle factors, such as maternal diet during pregnancy and lactation, delivery mode, timing of introduction to allergenic foods, farming lifestyle, animal exposure, family size, and the use of antibiotics have all been associated with risk of FA, highlighting pregnancy and the early postpartum period as critical windows to potentially modify FA risk. Utilizing results from birth cohorts, clinical trials, and other recent human studies, this review highlights microbial and immune biomarkers of FA and food sensitization (FS) during infancy and genetic, dietary, and environmental factors in early life that have been shown to influence risk for FA in early childhood.

Genetic Risk of Food Allergy

It has been well documented that the development of allergy carries a notable genetic component; however, there are varying risk levels. Although children with 1 parent with a history of allergic disease have double the risk of developing FA themselves, having 2 parents with allergic disease increases that risk to 3-fold.² Furthermore, FA in the index child has also been demonstrated as a significant and independent predictor of FA in other siblings (odds ratio, 2.6; 95% confidence interval, 1.2–5.6).⁷ For peanut allergies in particular, children with a parent or sibling with peanut allergy have been shown to have a 7-fold greater risk of developing peanut allergy than do children without a family history.⁸ However, it is plausible that some of the association identified between family history of FA and risk of FA in infancy may result from reverse causality owing to prolonged avoidance or late introduction of a particular food to the case infant. Recent data from Keet et al⁹ suggest that family history of peanut allergy alone, without the presence of moderate to severe eczema, did not confer substantial risk for peanut allergy during infancy. This finding does not totally discount a role for genetics in FA. Rather, the authors

raise the idea that genes involved with predisposition to peanut allergy may interact in some way with eczema susceptibility or manifest only after prolonged avoidance of peanuts and/or skin exposure.

Studies have begun to explore candidate genes associated with FA and FS. The major histocompatibility complex genes, which encode the human leukocyte antigen (HLA) complex, have been connected to FA development. Polymorphisms in Class II HLA genotypes DR and DQ have been linked to sensitization to peanut, cow's milk, and egg.^{2,8} Data from the Learning Early About Peanut (LEAP) trial demonstrate that these associations between genetic loci and immunologic responses to food antigens may differ in the presence or absence of oral exposure to a food. Among children in the trial with sustained oral exposure to peanut, the HLA-DQA*01:02 allele was related to higher immunoglobulin G4 levels specific for Ara h 2,¹⁰ whereas authors identified an association between carriage of *MALT1* risk alleles and peanut allergy among children who avoided peanut until 5 years of age.¹¹ Additional loci and their role as potential biomarkers in FA have been recently reviewed, including filaggrin (discussed below).² Beyond specific genetic variants, the regulation of gene expression by environmental and dietary exposures through epigenetic mechanisms has a demonstrated role in FA and other allergic diseases. Methylation profiles have been shown in multiple studies to discriminate children with and without FA, including peanut, egg, and cow's milk allergies,² and are likely a key mediator between environmental exposures and genetic risk.

Link Between Atopic Dermatitis and Food Allergy

About one-third of children with atopic dermatitis (AD) are susceptible to be diagnosed with immediate-type immunoglobulin (Ig)E-mediated FA.¹² The risk of developing allergy to cow's milk, eggs, and peanuts is increased in infants with early onset and severe AD.¹³ In addition, late introduction of peanuts into the diet of infants diagnosed with moderate to severe AD is associated with an increased risk of developing peanut allergy.⁹ It is posited that an impaired skin barrier in AD permits epicutaneous sensitization to foods before oral ingestion. This hypothesis is supported by studies demonstrating positive associations between environmental peanut exposure, measured by total household consumption or peanut levels in house dust, and risk of peanut allergy and sensitization.¹⁴ These associations are primarily found in populations at high genetic risk for AD. For example, increased risk of peanut allergy at school age has been associated with increased levels of peanut dust in an infant's play area; however, this relationship was only identified in children with null mutations in the filaggrin gene; there was no association in children with a normal genotype.¹⁴ Filaggrin is the main gene associated with skin barrier function, and loss-of-function mutations in this gene are associated with moderate and severe eczema.¹⁵ Mutations are thought to manifest as increased transepidermal water loss (TEWL), water loss across the stratum corneum, owing to increased skin barrier permeability. TEWL is higher in individuals with AD,¹⁴ and high levels can also precede clinical manifestation of AD¹⁵ and predict later FS.¹⁶ Compared to children with AD

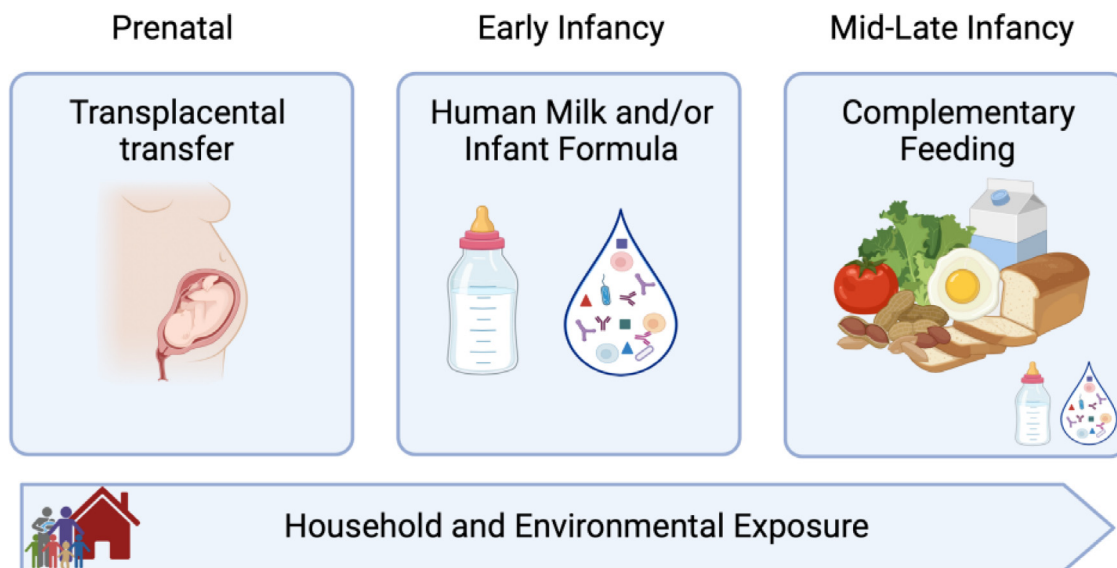


Figure 1. Routes of infant allergen exposure. In utero, food antigens may be transplacentally delivered to the fetus. After birth, infants can be exposed to food allergens through human milk or infant formula and eventually through their diet. Throughout these periods, individuals may also be exposed to allergens through their environment.

alone, the nonlesional skin of children with AD and peanut allergy has been characterized by increased TEWL, decreased filaggrin breakdown products, and altered lipid composition, despite similar skin severity scores in both groups.¹⁷ Given the associations between AD and FA, several studies have aimed at prevention of AD as a prevention strategy for FA as recently discussed.¹⁸ Findings on emollients as a primary prevention strategy are mixed, although they have demonstrated potential to reduce FS or FA. Breastfeeding and probiotic supplementation may protect against AD, but the data are inconclusive for prevention of FA. The association between vitamin D status, water hardness, and prebiotics, and synbiotics and incidence and/or severity of AD, FA, and FS have been explored; however, lack of randomized controlled trials (RCT), small sample sizes, and mixed findings have made it difficult to draw conclusions regarding their utility as a preventive strategy.¹⁸

Dietary Allergen Exposures in Early Life

Guidelines on early introduction of allergenic foods to reduce risk of FA have changed over the last 25 years. The most substantial departure from the status quo occurred because of the findings from the landmark study, the LEAP RCT, which demonstrated a protective effect of early introduction of peanuts against later peanut allergy.⁶ However, in this trial, many infants tested positive for peanut sensitization already at 4 to 6 months of age, suggesting allergen exposure before commencement of complementary feeding. There are multiple routes by which infants can be exposed to allergens in early life (Fig 1), although the mechanisms underlying sensitization or tolerance likely differ by means of exposure. Sensitization before introduction to solid foods could be a result of exposure to allergens in utero or through human milk or infant formula. However, sensitization by means of environmental and/or household exposure to allergens, particularly for infants with AD, is also plausible as milk and peanut levels in house dust have been demonstrated to elicit specific IgE responses in milk and peanut allergic individuals.¹⁴

Maternal Antigen Consumption During Pregnancy and Lactation

The existing literature on the relationship between maternal intake of allergenic foods and infant FA risk is slim, and findings are contradictory. Most food allergens have been detected in amniotic fluid,¹⁹ and this initial allergen encounter during fetal life is likely to play an

important role in initiating the development of tolerance or sensitization toward food allergens. Similarly, cow's milk, peanut, wheat, and hen's egg proteins have been detected in human milk, albeit at varying frequency and levels,²⁰ thus highlighting another potential means of exposure for the infant. The largest body of evidence related to maternal antigen consumption and later FA risk is related to peanut consumption,²¹ although results vary. Maternal ingestion of cow's milk during pregnancy and lactation has been shown to be protective against cow's milk sensitization or cow's milk allergy (CMA) in infancy.²² A few studies have assessed the relationship between maternal egg consumption and infant egg allergy risk, and although maternal egg ingestion may influence sensitization, no associations have been found between maternal egg consumption and clinical egg allergy. Table 1 summarizes recent studies. Many of the published studies on maternal dietary antigen consumption or avoidance possess limitations including potential recall bias, varying populations (nonatopic vs high-risk), differing clinical definitions, frequency and timing of perinatal antigen ingestion (pregnancy vs lactation), timing of dietary assessment (only assess during part of pregnancy or lactation), infant age of food introduction, and household exposure. No RCT results are available for peanut or milk, and more recent RCTs on egg, especially those that would be sufficiently powered, are lacking. Based on the current body of literature, the American Academy of Pediatrics (AAP) has concluded that there is not sufficient evidence to support maternal dietary restriction during pregnancy and lactation as a prevention strategy for atopic disease.²³

Breast- and Formula Feeding

Human milk (HM) is recognized for its beneficial impact on both maternal and infant health; however, findings from studies evaluating the protective effect of breastfeeding against FA are mixed.^{20,21} Although a recent clinical report from the AAP concluded that exclusive breastfeeding for at least 3 months is protective against early wheeze and decreases risk for AD, data were insufficient to suggest whether breastfeeding prevents or delays FA.²³ Differing definitions of breastfeeding and the actual composition of HM likely contribute to the contradictory findings. Alongside nutrients necessary for proper growth and development, HM contains several immunomodulatory components, which shape the early life microbiome and immune system.^{20,21} These components vary among women because

Table 1
Maternal Diet Containing Allergenic Foods and Its Association With Allergy or Sensitization in Offspring

Reference	Design	Outcome	Main result	Potential bias, limitations
Pitt et al, ⁶⁰ 2018, Canada	Secondary analysis of nested cohort of high-risk children, n = 342	Peanut sensitization at 7 y	Interaction analyses controlling for study group and maternal atopy: maternal peanut consumption while breastfeeding and infant peanut consumption by 12 mo were protective in combination, whereas either exposure in isolation was associated with an increased risk of sensitization (<i>P</i> interaction = .003).	Mothers who responded “Yes” at least once while breastfeeding (2 wk, 2, 4, 8, 12, 18, or 24 mo) were considered to have consumed peanut while breastfeeding. Number of cases is small when divided into subgroups. Ingestion during pregnancy or household exposure was not assessed.
Bunyavanich et al, ⁶¹ 2014, United States	Longitudinal prebirth cohort. General population, n = 1277	Peanut allergy at mean age 7.9 y (slgE > 0.35 kU/L and Rx of EpiPen or parent report of allergic reaction)	Higher maternal peanut intake during the first trimester was associated with 47% reduced odds of peanut allergic reaction (OR, 0.53; 95% CI, 0.30–0.94).	FFQ assessed first and second trimester only.
Tuokkola et al, ²² 2016, Finland	Prospective birth cohort at risk for type 1 diabetes, n = 4921 for analysis on pregnancy, n = 2820 for lactation.	CMA by 2 y	Highest quarter of cow's milk consumption during pregnancy was associated with lower risk of CMA in offspring. High intake of cow's milk only during pregnancy was associated with lower risk of CMA in offspring (OR, 0.30; CI, 0.13–0.68) when pregnancy and lactation were considered simultaneously. When stratified by maternal allergy risk, the association only persisted in offspring of nonallergic mothers (OR, 0.30; CI, 0.13–0.69).	Population at high risk for type 1 diabetes (HLA-conferred susceptibility) limits generalizability. Children whose CMA began before the maternal FFQ during lactation was completed were not included in the analysis. Adjustment for maternal allergy could only be performed in families who were still enrolled in study at 5 y.
Stråvik et al, ⁶² 2020, Sweden	Prospective birth cohort. General population, n = 508	FA at 1 y defined as an immediate or late-onset reaction to a specific food with prompt clinical improvement when food was removed from diet.	Higher intake of dairy products at 4 mo postpartum (<i>P</i> < .001) and cheese intake during pregnancy (<i>P</i> < .05) were associated with a lower prevalence of FA.	Dietary intake data were collected at 8 mo gestation and 1 and 4 mo postpartum, but the FFQ used only assessed food intake during the previous month. Infant food introduction/intake was not included in analysis.
Loewen et al, ⁶³ 2021, Canada	Prospective birth cohort. General population, n = 3412	SPT to food allergens at 1 and 3 y. Egg and peanut allergy at 3 y.	Maternal daily egg consumption during pregnancy was associated with child egg sensitization at 1 and 3 y but not egg allergy after accounting for moderate to severe atopic dermatitis, race, timing of infant egg introduction (1 y- OR, 2.64; CI, 1.44–4.83; 3 y – OR, 4.12; CI, 1.76–9.63).	Amount of egg consumed by mothers was not examined. Maternal diet during lactation was not accounted for.

Abbreviations: CI, confidence interval; CMA, cow's milk allergy; FA, food allergy; FFQ, food frequency questionnaire; OR, odds ratio; SPT, skin prick test.

of differences in maternal diet, environmental exposures, geographic location, genetics, and maternal health status,^{20,21} all factors that also influence allergic disease risk. Variations in and combinations of HM components may contribute to different immune trajectories of infants; however, this is not accounted for in epidemiologic studies. For example, the profile of human milk oligosaccharides (HMOs), nondigestible carbohydrates that shape the gut microbiome and modulate epithelial gene expression, has been associated with allergic sensitization and CMA.²⁰ Moreover, higher levels of both total IgA and cow's milk-specific IgA are also associated with protection against CMA.²⁴ As previously mentioned, food proteins can be secreted into HM after maternal ingestion.²⁰ Secretion may be affected by maternal intestinal permeability, quantity and/or frequency of antigen consumption, food preparation, and interindividual variation in metabolism, and not all mothers secrete dietary antigens after consumption.²⁰ In the cases where antigens are present in HM, perhaps the combined delivery of dietary antigens along with immunoglobulins may be protective against FA. Infants may also be introduced to cow's milk protein through cow's milk-based infant formula. Association between cow's milk formula exposure and FA risk varies greatly depending on the study. A recent systematic review concluded with low certainty that regular consumption of cow's milk formula throughout infancy is not associated with later risk for CMA in general or high-risk populations.²⁵ The group also concluded that temporary supplementation of cow's milk formula in the first week of life may increase risk of CMA, although this was

based only on 1 clinical trial. Conversely, a more recent clinical trial in Japan identified a decreased risk for CMA in infants who consumed at least 10 mL of cow's milk formula per day along with HM between 1 and 2 months of age.²⁶

Introduction to Solid Foods

Over the last 10 years, multiple clinical trials have been executed to investigate how early introduction of allergenic foods influences FA risk. Perhaps the most influential has been the LEAP trial, which was conducted among 640 high-risk infants in the United Kingdom. Trial findings demonstrated that early introduction (4 to 11 months) and sustained weekly intake of peanut until age 5 was protective against peanut allergy.⁶ Around the same time, the Enquiring About Tolerance (EAT) Study was conducted among 1303 breastfed infants from the general population in the United Kingdom to evaluate timing of introduction of multiple allergenic foods. Risk of FA was compared between infants who were introduced to peanut, cooked egg, cow's milk, sesame, whitefish, and wheat at either 3 months (early) or 6 months of age (standard).²⁷ Although results from the intention-to-treat analysis were not different between groups, the per-protocol analysis showed that infants in the early introduction group had a 67% lower risk of FA at 1 to 3 years of age than those in the standard group. The risks for peanut and egg allergies were also lower in the early introduction group, although protection appeared to be

dependent on amount and duration of exposure to the food. Findings primarily from the LEAP study, but also the EAT and LEAP-on trials, prompted the National Institute of Allergy and Infectious Diseases to publish new guidelines for peanut introduction based on presence and severity of eczema and other food allergies.²⁸ Conclusions from the AAP regarding general introduction of allergenic foods have not changed because the current evidence does not support delaying allergenic foods beyond 4 to 6 months for prevention of atopic disease.²³

In addition to the EAT study, several recent clinical trials have addressed the effect of early egg introduction on FA or FS and have resulted in mixed findings. Early introduction of uncooked egg white powder to infants from the general population showed no protection against IgE-mediated FA and/or sensitization at 1 year²⁹; similar results were found for pasteurized, raw whole egg powder in a group of high-risk infants.³⁰ However, in other trials, early introduction of uncooked egg white powder was associated with a reduction in egg white sensitization,³¹ and whole, heated egg powder administered in a step-wise manner was protective against oral food challenge diagnosed egg allergy at 12 months.³² Based on current evidence, the American Academy of Allergy, Asthma & Immunology, the American College of Allergy, Asthma, and Immunology, and the Canadian Society of Allergy and Clinical Immunology recommend introduction of cooked egg or egg-containing products to all infants around 6 months of life as an approach for prevention of egg allergy.³³ By contrast, the current stance of the AAP is that more studies are necessary to confirm whether early egg introduction would in fact reduce risk of egg allergy.²³

Lifestyle and Environmental Exposures

Although there is certainly a genetic component to FA and FS, shifts toward industrialization and adoption of a Westernized lifestyle over the last half century are hypothesized to contribute to the appreciable rise of FA and other allergic diseases.³ The “hygiene hypothesis,” proposed by Strachan et al in 1989, was based on the finding that a larger family size was related to a lower incidence of AD and hay fever.³⁴ Since this observation, many studies have highlighted the role of microbial exposure in immune development and risk of FA and other atopic diseases during childhood. Maternal and child exposure to pets and vaginal vs cesarean delivery³⁵ have been associated with a lower risk for allergic disease, whereas antibiotic use in early life is a known risk factor.³⁶ A strong body of literature has also demonstrated a low prevalence of FA and other allergic diseases among individuals who follow a traditional, single-family farming lifestyle,^{37,38} although the degree of protection does vary among even seemingly quite similar, traditional farming populations.³⁹ Besides number of siblings, consumption of unpasteurized cow's milk and contact with farm animals and stables are specific aspects of the farming lifestyle that have been identified to be protective against asthma, hay fever, and atopic sensitization.^{37,38} The consistent exposure to a diverse profile of microorganisms in early life may modify allergy risk by training the immune system to mount a tolerogenic response upon later exposure to environmental or food antigens.³⁶

Gut Microbiome and Metabolome in Food Allergy

The role of the gut microbiome in immune development and programming during early life is well documented, and substantial pre-clinical and clinical evidence points to the early gut microbiome as a factor mediating the association between industrialization and increases in allergic disease prevalence.⁴⁰ To date, multiple studies have demonstrated relationships between the early fecal microbial profile and risk of FA and FS in childhood (Table 2 summarizes recent studies). Reports vary on microbial diversity and taxonomic composition during infancy and association to later FA and FS. Greater

microbial diversity is regarded as beneficial during adulthood. However, this measure may be less straightforward in early infancy because breastfeeding, which is regarded as favorable for microbial development, is typically associated with a less diverse microbiome. Similarly, the taxonomic signature associated with FA or FS differs among studies, perhaps because of differences in geography, timing of sample collection, and/or clinical diagnostic criteria. However, studies do suggest that development of FA or FS may be associated with a less mature microbiome, marked by a high Enterobacteriaceae/Bacteroidaceae ratio⁴¹ and low microbiota-for-age Z-score⁴² during the first year of life. Clostridia, a bacterial class within the Firmicutes phylum, appears to have a role in protection against FA or FS development, findings which reflect those in preclinical models.⁴⁰ Clostridiales was lower at 6 months in infants who developed FA,⁴² and the genus *Clostridium* was identified to be lower in infants who went on to develop atopic sensitization.⁴¹ The fecal microbiota at 3 to 6 months in infants whose CMA resolved by 8 years of age was also shown to be enriched with *Clostridium* compared with infants whose allergy did not resolve.⁴³

Functional capacity may be redundant among members of the microbiome, which leads to the question of whether differences in taxonomy identified between atopic and nonatopic infants equate to differences in functional capacity of the microbiome (functional genes) and of course functional output (metabolites). Along with taxonomic differences, infants in the CHILD cohort who developed atopic sensitization were found to harbor fewer genes related to HMO metabolism, complex polysaccharide degradation, and butyrate synthesis in their fecal microbiome at 3 months.⁴⁴ Butyrate levels have in fact been shown to be lower in stool of infants with CMA at around 1 year of age.⁴⁵ Furthermore, infants with the highest levels of fecal butyrate were less likely to demonstrate atopic sensitization or report FA in the PASTURE/EFRAIM cohort; high levels of propionate were also associated with a decreased risk for atopic sensitization.⁴⁶ Butyrate and propionate are short-chain fatty acids (SCFAs), which are the most abundant microbial metabolites in the colon, produced predominantly from fermentation of dietary fiber and other nondigestible carbohydrates, such as HMOs. Through a variety of mechanisms, SCFAs maintain barrier function and promote a tolerogenic environment within the gastrointestinal tract, which may aid in protection against FA.⁴⁷ We have previously identified that infants in the Old Order Mennonite community, who follow a traditional farming lifestyle, have substantially higher prevalence and abundance of *Bifidobacterium longum* subspecies *infantis* (*Bifidobacterium infantis*) than suburban infants at high risk for allergic disease.⁴⁸ In addition, *B. infantis* also tended to be lower among infants, who went on to develop atopic disease (FA and/or AD) by 3 years of age. A recent study by Henrick et al showed that infants supplemented with *B. infantis* EVC001 had 10-fold higher fecal levels of indole-3-lactic acid than nonsupplemented infants at 3 weeks.⁴⁹ Indole-3-acetic acid, a tryptophan metabolite, has been demonstrated to decrease enteric inflammation in infants and exert regulatory effects on Th2 and Th17 cells,⁴⁹ highlighting means by which *B. infantis* may influence early immune development. Higher fecal levels of several proinflammatory metabolites, in particular 12, 13-diHOME, have also been identified among infants at greatest risk for multisensitized atopy.⁵⁰ Taken together, taxonomic differences between atopic and nonatopic infants differ among studies, but the functional capacity and metabolic signatures of FA and FS seem more consistent and are likely more useful biomarkers of disease risk. However, to the best of our knowledge, no published studies investigating associations specifically between FA and microbiome composition have yet employed shotgun metagenomic analysis techniques, thus limiting taxonomic resolution and information about metabolic capacity of the microbiome. Metagenomic and complementary metabolomic analyses are necessary to advance our understanding of host–microbe interactions in the context of FA and FS.

Table 2
Associations Between Infant Fecal Microbiota Composition and Food Allergy or Sensitization Outcomes

Reference	Sample size	Sample time point	Outcome	Diversity	Taxonomy
Azad et al, ⁶⁴ 2015, Canada	166	3 and 12 mo	FS at 12 mo	FS had decreased gut microbiota richness at 3 mo than did HC. Significant difference in overall composition between FS and HC at both time points. At 3 mo, each quartile increase in gut microbiota richness was associated with a 52% reduction in risk for FS by 1 y.	• FS infants were enriched with Enterobacteriaceae at 3 and 12 mo and had decreased Bacteroidaceae and Ruminococcaceae at 3 mo. • FS infants had higher Enterobacteriaceae/Bacteroidaceae (E/B) ratio at 3 and 12 mo. • Each quartile increase in E/B ratio was associated with a 2-fold increase in risk for food sensitization.
Savage et al, ⁶⁵ 2018, United States	216	3–6 mo	FS or FA at 3 y	Alpha diversity was not different between any groups.	• Read counts of <i>Dorea</i> (Clostridia class), <i>Dialister</i>, <i>Clostridium</i> (Clostridia class), and <i>Haemophilus</i> were lower among FS infants. • Read counts of <i>Dorea</i> (Clostridia class), <i>Citrobacter</i>, <i>Oscillospira</i> (Clostridia class), and <i>Lactococcus</i> were lower in FA infants.
Petersen et al, ⁴¹ 2021, Canada	950	3 and 12 mo	Sensitization to food and/or aeroallergens at 12 mo	HC had a richer microbiota at 3 mo.	• Sensitized infants had a less mature microbiota at 3 mo. • Multiple taxa were differentially abundant between groups at 3 mo. HC were enriched with <i>Coprococcus</i> (Clostridia class), <i>Blautia</i> (Clostridia class), <i>Ruminococcus</i> (Clostridia class), Lachnospiraceae (2 unclassified genera; Clostridia class), Peptostreptococcaceae (2 unclassified genera), <i>Clostridium</i> (Clostridia class), <i>Eubacterium</i> (Clostridia class), <i>Eggerthella</i>, and <i>Oscillospira</i> (Clostridia class). • Enterobacteriaceae were higher in infants who had positive SPT at 3 and 12 mo.
Fujimura et al, ⁵⁰ 2016, United States	308	1 and 6 mo	Multisensitized atopy at 2 y and/or asthma at 4 y.	Overall composition was associated with neonatal gut microbiota composition states.	• Infants in the microbiota composition state with the greatest relative risk of multisensitized atopy and asthma had an early gut microbiota profile depleted in <i>Bifidobacterium</i>, <i>Lactobacillus</i>, <i>Faecalibacterium</i> (Clostridia class), and <i>Akkermansia</i> and a higher abundance of <i>Candida</i> and <i>Rhodotorula</i>.
Tanaka et al, ⁶⁶ 2017, Japan	56	1, 2, 6, 12 mo	FA by 2 y; other atopic disease by 3 y.	Phylogenetic diversity was lower among FA at 1 y, and richness also tended to be lower among FA at 1 y. Alpha diversity did not differ between FA and HC at 1 or 6 mo. Least square discriminant analysis, but not UniFrac distance, showed differences in microbial structure between FA and HC at 2 mo and 1 y.	• <i>Leuconostoc</i>, <i>Weissella</i>, and <i>Veillonella</i> were lower at 2 mo in infants who developed FA. • <i>Clostridium</i> (Clostridia class) and unclassified Enterobacteriaceae were enriched at 1 y in FA infants. • abundance of <i>Clostridium paraputrificum</i> (Clostridia class) and <i>Clostridium tertium</i> (Clostridia class), as assessed by qPCR, was higher in FA at 1 y.
Joseph et al, ⁴² 2022, United States	447	1 and 6 mo	FS or FA by 5 y.	Those with IgE-mediated FA between 3 and 5 y showed reduced alpha diversity at 6 mo compared with HC. FS and FA were associated with overall microbiome composition at 6 mo with covariate adjustment.	• Several taxa, primarily from Lactobacillales, Bacteroidales, and Clostridiales (Clostridia class) orders were lower in FA infants at 6 mo compared with HC. • Bifidobacteriales was enriched at 6 mo in FA infants at 6 mo. • FA infants had a lower microbiota for age Z-score than HC.
Bunyavanich et al, ⁴³ 2016, United States	226	3–16 mo	Cow's milk allergy	Overall microbiome composition at 3–6 mo differed between those who did and did not resolve milk allergy by 8 y - only at earliest sampling. Trend for greater bacterial diversity among subjects whose allergy resolved.	• Microbiota of those whose milk allergy resolved by 8 y was enriched with Clostridia class and Firmicutes. • Bacteroidetes and <i>Enterobacter</i> were enriched in microbiota of infants whose allergy did not resolve.
Fazlollahi et al, ⁶⁷ 2018, United States	141	3–16 mo	Egg allergy and sensitization	Children with egg allergy had a distinct and more diverse gut microbiota. Egg sensitization also associated with a distinct gut microbiota and increased alpha diversity.	• <i>Ruminococcus</i> (Clostridia class) and <i>Lactococcus</i> abundances were enriched in egg allergic infants. • <i>Roseburia</i> (Clostridia class) and <i>Faecalibacterium</i> (Clostridia class) were enriched in egg sensitized infants.
Berni Canani et al, ⁶⁸ 2016, Italy	39	1–12 mo	Cow's milk allergy	FA infants had a more diverse microbiome than HC.	• <i>Leuconostoc</i> was enriched in HC. • At the genus level, FA infants had enriched <i>Ruminococcus</i> (Clostridia class) and <i>Faecalibacterium</i> (Clostridia class) and lower abundances of <i>Bifidobacterium</i> and <i>Escherichia</i> compared with HC.
Seppo et al, ⁴⁸ 2021, United States	78	2 mo (median)	FA and/or atopic dermatitis by 3 y.	No difference in alpha diversity between atopic and nonatopic infants.	• Atopic infants had higher abundances of <i>Lactobacillus</i>, <i>Bacteroides</i>, and <i>Eggerthella</i> than nonatopic infants.

Abbreviations: FA, food allergy; FS, food sensitization; HC, healthy controls; IgE, immunoglobulin E; SPT, skin prick test.

Table 3
Immune Biomarkers in Food Sensitization and Food Allergy

Cell type	Age	Food allergy or sensitization	Results	Reference
Monocyte	1 y	Egg	- Increased monocyte: CD4⁺ T cell ratio in allergic infants - Increased cytokine (IL-6, IL-6, IL-1β) production following LPS stimulation in allergic infants	Neeland et al, ⁵¹ 2020, Australia
	Cord blood	Sensitization to cow's milk, egg, peanut, sesame, cashew at 1 y	Increased cord blood monocyte: CD4⁺ T cell ratio in sensitized infants	Zhang et al, ⁶⁹ 2016, Australia
	1 and 2–4 y	Egg	- Increased circulating monocytes in persistent egg allergic infants - Increased cytokine production following LPS stimulation in persistent egg allergic infants	Neeland et al, ⁵⁷ 2018, Australia
T cells	1 and 2–4 y	Egg	- Hypoproliferative response in naïve CD4⁺ T cells of allergic infants following CD3/CD28 stimulation - gene expression and methylation analysis revealed an intrinsic defect in the T cell activation response	Martino et al, ⁵² 2018, Australia
	1 y	Peanut	- Increased TNFα⁺ cells in peanut allergic following PMA/ionomycin stimulation - Increased CD40L⁺CD69⁺ T cells in peanut allergic compared with peanut sensitized tolerant following peanut protein stimulation	Neeland et al, ⁵¹ 2020, Australia
	1 y	Peanut	- Increased IL-13⁺CD154⁺ effector T cells in sensitized and allergic infants following stimulation with crude peanut extract - Increased skin-homing CLA⁺, decreased gut-homing α4β7⁺ CD154⁺ effector T cells in sensitized and allergic infants following stimulation with crude peanut extract	Weissler et al, ⁷⁰ 2018, Australia, United States
Regulatory T cells	Cord blood	Sensitization to cow's milk, egg, peanut, sesame, cashew at 1 y	Decreased cord blood regulatory T cells with less FOXP3 demethylation in sensitized infants	Zhang et al, ⁶⁹ 2016, Australia
	3–18 mo	Cow's milk	Low FoxP3 TSDR methylation in allergic infants	Paparo et al, ⁷¹ 2016, Italy
	1 y	Egg	Decreased naïve regulatory T cells in allergic infants	Neeland et al, ⁵¹ 2020, Australia
	1–15 mo	Cow's milk, soy, egg, tree nuts, fish, shellfish, wheat, peanuts	Decreased RORγt⁺ regulatory T cells in allergic infants	Abdel-Gadir et al, ⁵⁴ 2019, United States
B cells	1 y; follow-up	Egg	- Decreased B cell subsets and IL-8 following CpG stimulation in allergic infants - Decreased switched and nonswitched B cells and altered cytokine response in persistent allergic infants	Neeland et al, ⁵⁶ 2019, Australia
	1 y	Peanut	Increased CD19⁺HLA-DR⁺ cells in peanut allergic infants	Neeland et al, ⁵⁶ 2019, Australia
slgA and IgE	1–15 mo	Cow's milk, soy, egg, tree nuts, fish, shellfish, wheat, peanuts	Decreased slgA and increased IgE binding to fecal microbes in allergic infants	Abdel-Gadir et al, ⁵⁴ 2019, United States
IgG and IgE	Cord blood and 6 mo	Egg (ovomucoid)	- Decreased cord blood ovomucoid specific IgG1, IgG2, and IgG4 in infants who were later sensitized at 6 mo - Negative correlation between ovomucoid cord blood IgG4 and IgE at 6 mo	Irahara et al, ⁵⁵ 2019, Japan
IgE	6 mo–4 y	Egg white, cow's milk	- Association between total IgE and egg white or cow's milk specific IgE at 6 mo, 1, 1.5, 2, and 4 y of age - Higher prevalence of food sensitization in children with total IgE levels >100 kU/L (6 mo–4 y)	Wong et al, ⁷² 2020, Taiwan

Abbreviations: CLA, cutaneous lymphocyte-associated antigen; CpG, cytosine-phosphate-guanosine; IgA, immunoglobulin A; IgE, immunoglobulin E; IgG1, immunoglobulin G1; IgG2, immunoglobulin G2; IgG4, immunoglobulin G4; IL, interleukin; kU/L, kilounits per liter; LPS, lipopolysaccharide; slgA, secretory immunoglobulin A.

Potential Early Immune Biomarkers of Food Allergy

The immune system rapidly develops in early life. Immune cells related to innate and adaptive immunity are present before birth and have been shown to be responsive to environmental cues. Besides specific IgE sensitization, several studies have demonstrated that FS and FA are associated with a differential infant immune profile. The profile has been reported to include increased production of proinflammatory and Th2 cytokines,^{51,52} emergence of activated T and B cell populations,⁵³ altered IgG and IgA responses,^{54,55} and even the loss of regulatory T cells.⁵¹ Moreover, reports have demonstrated that besides differing from nonallergic infants, the immune profile among infants with persistent FA and those where FA has resolved (transient) may differ as well.^{56,57} Although they have been described in older children and adults, additional T cell populations like pathogenic effector Th2a cells⁵⁸ and peanut specific CD154⁺ T cells⁵⁹ have been found elevated in peanut allergic individuals. Studies on the presence and/or emergence of these cells in infant populations is lacking. A summary of recent studies on early life immune findings in the context of FS and FA can be found in Table 3. It is not currently understood how these responses have mechanistically developed. A potential hyperresponsive profile combined with the loss of immune cell populations, such as those with an immunoregulatory function, could increase the risk for aberrant responses including sensitization.

Conclusion

Although genetics play an obvious role in FA development, maternal and infant allergen exposure, HM composition, and other environmental factors are all associated with risk for FA, highlighting a complex network of factors that influence disease pathogenesis (Fig 2). The collective effects of these exposures on FA risk are likely mediated through their effects on multiple biological targets in the infant, such as the early microbiome and immune system, potentially through epigenetic mechanisms. Clinical trials have demonstrated that early introduction of allergenic foods can be protective against later allergy, but it is evident that sensitization can happen before complementary food introduction. Infants may be exposed to antigens in utero, through HM or infant formula, or through environmental exposure. Tolerance or sensitization likely depends on the route of first exposures and perhaps genetic risk. However, to the best of our knowledge, no studies have comprehensively measured potential antigen exposure, and food-specific antibodies delivered to the infant, across the pre- and postnatal periods. Most literature has focused on associations between maternal dietary intake during a limited timespan (ie, just pregnancy or lactation) and without assessment of the level of exposure (such as the amount of dietary allergens in mother's milk). Although this information is necessary to

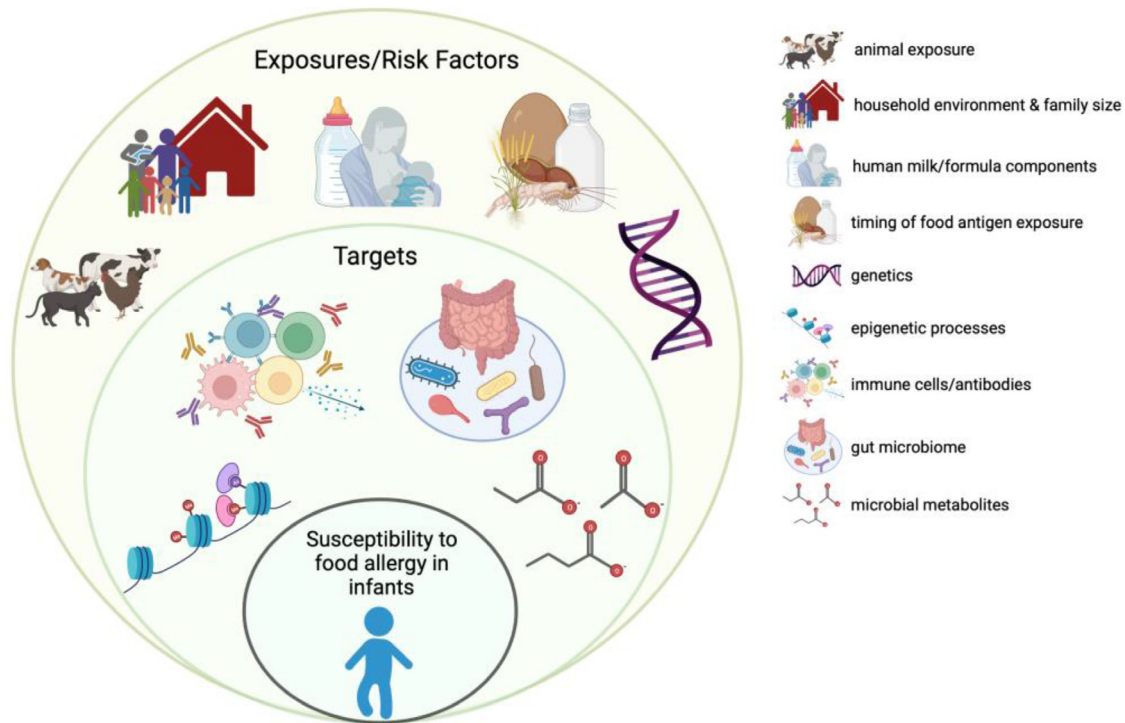


Figure 2. Early life risk factors and targets that influence susceptibility to food allergy. Genetics, animal exposures, family size, breastfeeding, and early allergen exposure are associated with food allergy risk. These factors are hypothesized to influence risk through effects on the microbiome, metabolome, and the developing immune system, potentially through epigenetic mechanisms.

develop dietary guidelines during pregnancy and lactation, maternal dietary intake data alone do not account for interindividual variation in metabolism or account for environmental exposure in the home.

The infant fecal microbiome and metabolite profiles are associated with FA and FS. Although there does seem to be a specific gut microbiome signature of FA, the current body of literature mostly comprises studies conducted using 16S rRNA sequencing, which limits taxonomic resolution and provides no information on functional capacity of an individual's microbiome. To develop novel microbiome-targeted prevention strategies, more studies employing metagenomic analysis techniques and performing large-scale assessment of the fecal metabolite pool are needed. Furthermore, there is a need for earlier and longitudinal sampling of the microbiome because symptoms of FA can manifest early in infancy through toddlerhood. Existing literature demonstrates some immune biomarkers associated with FA and FS, but few studies have focused on the development of specific antibody responses in early life or investigated longitudinal innate and adaptive responses between atopic and nonatopic infants. Moreover, continued investigation into the differential responses in infants who are nonallergic, sensitized nonallergic, have IgE-mediated FA, and those in whom allergy has resolved is warranted. This current body of evidence highlights the first 1000 days of life as a critical window for FA development and emphasizes the need for additional observational studies and clinical trials spanning early pregnancy through childhood to uncover novel biomarkers and risk factors that can predict susceptibility toward or protect against FA.

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