Potential biomarkers, risk factors and their associations with IgE-mediated food allergy in early life: a narrative review

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Abbreviation	Meaning
CHILD	Canadian Healthy Infant Longitudinal Development
CMA	Cow's milk allergy
CRD	Component-resolved diagnostic
DBP	Vitamin D binding protein
EAACI	European Academy of Allergy and Clinical Immunology
EAT	Enquiring about tolerance
FA	Food Allergy
HLA-DQ	Human leukocyte antigen DQ isotype
HLA-DR	Human leukocyte antigen DR isotype
OFC	Oral food challenge
OIT	Oral immunotherapy
RCT	Randomized control trials
SERPINB	Clade B serpin
SPT	Skin prick test
Treg	T-regulatory

Abstract

Food allergy affects the quality of life of millions of people worldwide and presents a significant psychological and financial burden for both national and international public health. In the past few decades, the prevalence of allergic disease has been on the rise worldwide. Identified risk factors for food allergy include family history, mode of delivery, variations in infant feeding practices, prior diagnosis of other atopic diseases such as eczema, and social economic status. Identifying reliable biomarkers which predict the risk of developing food allergy in early life would be valuable in both preventing morbidity and mortality and by making current interventions available at the earliest opportunity. There is also the potential to identify new therapeutic targets. This narrative review provides details on the genetic, epigenetic, dietary and microbiome influences upon the development of food allergy and synthesizes the currently available data indicating potential biomarkers. While there is a large body of research evidence available within each field of potential risk factors, there are very limited number of studies which span multiple methodological fields, for example including immunology, microbiome, genetic/epigenetic factors and dietary assessment. We recommend that further collaborative research with detailed cohort phenotyping is required to identify biomarkers, and whether these vary between at-risk populations and the wider population. The low incidence of oral food challenge confirmed food allergy in the general population, and the complexities of designing nutritional intervention studies will provide challenges for researchers to address in generating high quality, reliable and reproducible research findings.

Keywords: IgE mediated food allergy, biomarkers, pathways, risk factors, microbiota, nutrition, infant diet

Statement of significance

Food allergy affects the quality of life of millions of people worldwide and presents a significant psychological and financial burden for both national and international public health. Identifying reliable biomarkers which predict the risk of developing food allergy would be valuable in both preventing morbidity and mortality and by making current interventions available at the earliest opportunity. This review provides details on the genetic, epigenetic, dietary and microbiome influences upon the development of food allergy. This helps in identifying reliable biomarkers to predict the risk of developing food allergy, which could be valuable in both preventing morbidity and mortality and by making interventions available at the earliest opportunity.

1. Introduction

Food allergy (FA) is defined as an adverse immunologic response to a food protein (1). It affects the quality of life of millions of people worldwide and presents a significant psychological (2) and financial (3) burden for both public health. The European Academy of Allergy and Clinical Immunology (EAACI) systematic review estimates FA prevalence in Europe between 0.1 and 6.0% (4). Risk factors for developing FA are multiple and contextual, ranging from genetic predisposition to environmental factors (such as mode of birth delivery, type and timing of solid food introduction, changes in hygiene practices, and social economic status) and the interaction between these factors (**Table 1**).

Identifying biomarkers that reflect either the risk of developing FA, the severity of FA or induction of tolerance (i.e. reaching non-reactivity towards a substance that would previously cause a reaction) would be valuable in both preventing morbidity and mortality arising from FA, by allowing earlier interventions and by potentially highlighting new targets for intervention. The Health Biomarkers Definitions Working Group defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (5).

Biomarkers can also provide value in the regulatory context. The European Food Safety Authority health claim substantiation requires that "a food or one of its constituents significantly reduces a risk factor in the development of a human disease" (6). The regulation additionally requires that the risk factor is `generally accepted`. A classic example is cholesterol, a biomarker found to be associated with heart disease development. In labelling or advertising, health claims which constitute a "reduction of disease risk" shall also bear a statement indicating that the disease to which the claim is referring has multiple risk factors and that altering one of these risk factors may or may not have a beneficial effect. Thus, the

optimal risk biomarker to be altered would be a combination of risk factors or a chain of events reflecting changes in the relative risk.

This paper reviews available evidence in human studies in early life about well-described pathways with well-defined biomarkers and risk factors which are associated with IgE-mediated FA.

2. Current status of knowledge

Recent efforts have focused on the identification of biomarkers for prediction and diagnosis of IgE-mediated FA. IgE-mediated reactions induce a variety of symptoms that range from erythema, urticaria and angioedema, nausea, abdominal pain or vomiting, to severe respiratory distress, or cardiovascular collapse among others (7). Differences in the outcomes and manifestations might be related with genetic components but also with environmental factors, dietary factors and the intestinal microbiota (8). The exact diagnosis and prevalence of FA is difficult to ascertain due to the imprecision of laboratory tests and the lack of specific biomarkers, relying on the combination of the clinical history of characteristic symptoms together with test results (7), the use of IgE as a biomarker in FA, and the potential associations with genetic and epigenetic origins that would be target of potential interventions (breast milk versus others, weaning, diet, etc.).

2.1. Genetic and epigenetic biomarkers of food allergy

The link between the risk of FA in children and allergic diseases and/or allergic sensitisation in their family has been extensively reported (9-14), with estimates that FA/sensitization risk doubles if one parent has an allergic disease and is threefold higher if both parents having an allergic disease. A recent meta-analysis of genome-wide association studies identified 10 loci near *C11orf30* (encoding the transcriptional repressor EMSY), *STAT6*, *SLC25A46*, *HLA-DQB1*, *IL1RL1* (encoding ST2, the β-chain of the IL-33 receptor), *LPP*, *MYC*, *IL2*, and *HLA-*

B that are associated with allergic sensitisation (15). Allergen-specific genetic modifications in the human leukocyte antigen (HLA) DR and DQ isotype gene region have also been associated with peanut allergy (16). Conflicting results were reported with regards to gender association with FA and no conclusive studies are available (10, 11, 17, 18). Some data suggests that five loci at genome-wide significance (clade B serpin [SERPINB]) gene cluster at 18q21.3, the cytokine gene cluster at 5q31.1, the filaggrin gene, the C11orf30/LRRC32 locus, and the HLA region increase the risk for FA (19).

Eczema and FA often co-exist and evidence suggests that impaired skin barrier is a significant risk factor for FA development later in life (20, 21) with loss-of-function variants in the filaggrin gene suggested as a causative factor, and filaggrin mutation is associated with eczema and asthma later in life (22, 23). Identified genetic loci associated with FA, their potential mode of action and evidence supporting their use as a biomarker are presented in **Table 2**.

Extrinsic environmental factors including diet, pollutants and infections, and intrinsic factors such as the intestinal microbiota and inflammatory state are likely to play a crucial role in inducing epigenetic changes (24, 25). The importance of postnatal factors and environmental influence is a risk factor for FA development and this exposure accumulates while the infant develops (9, 10, 18). The route of exposure (e.g. placental, skin, breast milk, airway, gut), timing, dose of allergen exposure, and host immune system status are likely to impact upon the potential for epigenetic change (26). Investigations of targeted and untargeted methylation profiles of immune cells are methodologies that can help to find biomarkers that reflect the different stages of FA: those at risk, those who are tolerant, those with active disease (27, 28). An overview of studies on epigenetic changes associated with FA is presented in **Table 3**.

2.2. The role of breastfeeding, and time of food introduction in FA

2.2.1. Breastfeeding

Human milk is the first food available to a newborn baby and exclusive breastfeeding for the duration of 6 months is recommended by the WHO. Available evidence suggests that breastfeeding protects against infections as well as offers long-term benefits, reducing the risk of hypertension and diabetes, and improving cognitive development (29). The protective effect of breastmilk on allergy development has not been fully demonstrated (29-33). However, there is conflicting data concerning the relationship between breastfeeding and FA with some cohort studies reporting a reduced risk of FA development in the general population (20, 21) and in high-risk children (34) while others reporting an increased risk (35, 36). One meta-analysis investigating this relationship reported no evidence of breastfeeding's protective effect in preventing FA development (OR: 1.02; 95% CI: 0.88, 1.18), although the authors suggested that the risk of bias and major differences in the outcome definitions in the current studies may be responsible for the inconclusive results (31). As human milk contains food proteins, their levels in the milk and maternal diet may also contribute to tolerance development (37), particularly in presence of the biologically active molecules (38). Both aspects are not normally considered in the studies, assessing associations between breastfeeding and non-communicable diseases development.

A recent systematic review on FA prevention suggests that although breastfeeding has many benefits for infants and mothers, it may not reduce the risk of FA (39). Human breast milk constituents vary (over time postpartum, within and between women and even within the same feed), which may, in part, explain some of the conflicting results of general observational studies regarding the provision of breastfeeding (40, 41). It has been described that immunological compounds in breast milk (including cytokines and immunoglobulins) are modulated by multiple factors, including maternal allergic status, parity, geographical

location among others (42-45), but overall evidence on the topic is conflicting with most of the studies not identifying clear associations between the immunological composition of breast milk and allergic disease development in infants (38). Dietary peptides from proteins in food are excreted in breast milk, but the peptide sequences are relatively short and in small amount, therefore, their sensitization or tolerogenic potential remain to be explored (46). The presence of specific peptides has also been shown in infant formula (47). However, so far, systematic reviews (48, 49) have not found sufficient evidence that hydrolysed formula prevents eczema or milk allergy (50).

Thus, claims currently appearing on the infant formula products need better substantiation and many reputable organizations, including the American Academy of Pediatrics; American Academy of Allergy, Asthma, and Immunology; American College of Allergy, Asthma, and Immunology; Canadian Society for Allergy and Clinical Immunology concluded that "there is no protective benefit from the use of hydrolyzed formula in the first year of life against food allergy or food sensitization" (51, 52). A recent study suggested that avoiding temporary supplementation with conventional cow's milk formula in the first three days of life may result in a large decrease in the risk of food allergy in early childhood (53) but this requires further confirmation.

2.2.2. Weaning and food introduction

Delaying the introduction of solid food until 6 months remains the current WHO recommendation. Yet recent expert opinion has investigated the hypothesis that oral tolerance can be induced by modifying the timing and diversity of early food exposure (54). Supportive data for this hypothesis is coming predominantly from two large high quality randomised control trials (RCT) LEAP and Enquiring About Tolerance (EAT). The LEAP study demonstrated a significant reduction in peanut allergy prevalence in children at high risk of allergy development, who were consuming peanuts between 4 and 11 months old on a regular

basis (55). There was an earlier and greater increase in peanut specific IgG and IgG4 in the early consumption group compared to the avoidance group. In both groups the mean peanut specific IgE levels were highly comparable and increased over time with the note that there were more participants in the avoidance group with very high IgE levels (55). The EAT trial looked at early food introduction (from 3 months old) and concluded that it may decrease the risk of FA development (56). The authors reported significantly lower relative risks of peanut and egg allergy in the early-introduction group, with no difference in the prevalence of milk, sesame, fish, or wheat allergy. Risk reduction was shown in per-protocol analysis only, while no statistically significant difference was found in intention to treat analysis. Studies reporting contradicting results to EAT exist (57), but they are often considered of lower robustness.

With an apparent shift in expert opinion towards early introduction of certain highly allergenic foods, the American National Institute of Allergy and Infectious Diseases, updated its guidelines on peanut allergy prevention in 2017 (58), recommending that peanut-containing food introduction should occur between 4 and 6 months of age in egg allergic infants and/or babies with severe eczema and at 6 months of age for infants with mild-to-moderate eczema. Recent guidelines from the American Academy of Pediatrics support these recommendations (52).

In their systematic review on FA prevention, the authors concluded that available evidence suggests that "*introduction of a small amounts of cooked, egg into the infant diet as part of complementary feeding probably reduces the risk of egg allergy in infancy and in countries with a high prevalence of peanut allergy, introducing regular peanut consumption from 4-11 months of life in infants at increased risk probably results in a large reduction in peanut allergy in early childhood compared to completely avoiding peanut for the first five years"* (39). In contrast, no reduction in FA incidence was found when multiple potential food

allergens were simultaneously introduced into the infant diet as of three months (56). Diet diversity during the first year of life may also have a positive role in determining the risk of FA. An increased diversity of complementary foods introduced in the first 12 months of life was inversely associated with FA development up to 6 years old (59).

2.3. Is there a need of biomarkers to monitor dietary interventions to induce tolerance?

Food avoidance remains the main therapeutic approach in FA management, but researchers and clinicians are continuously seeking for intervention options. Controlled exposure to the allergens was suggested as a potential option for tolerance induction. Indeed, in recent years, Oral immunotherapy (OIT) has been applied for several allergens to investigate whether desensitisation and/or sustained unresponsiveness development is possible. A meta-analysis on the effect of OIT in reducing prevalence of CMA concluded it is an effective therapy (60); however, frequency of adverse events is high and validity of outcome selection used to measure the efficacy of OIT is still unclear. Looking at an individual study level, there was no association of OIT in children (6-17 yo) and IgE levels between the treated and the control group, whereas IgG4 was significantly increased in the post-treatment group after OIT but there was only a slight increase in the control group (61). Recently, a cohort of 137 peanut allergic child and adult patients (6-26 yo) were compared to non-peanut allergic controls and examined differences between IgE, IgG4 and the ratio of IgG4/IgE (62).

These observations would imply that more data are needed on sIgE and IgG4 in monitoring tolerance induction over time before being able to conclude that these are reliable biomarkers for tolerance induction. There may be more potential for the increase in IgG4 in oral tolerance induction than the decrease in IgE. It is very important to note that there are no agreed core outcome measures in FA trials, which do not allow for appropriate

effectiveness/efficacy evaluation (63). Different immunological parameters are currently used as endpoints in OIT trials, but available evidence of their importance is very limited (64).

3. What is the role of the microbiota in food allergy?

A link between IgE-mediated FA and the gut microbiota composition and metabolic activity has been suggested. A recent study including 233 infants (> 4 years old) with FA (milk, sesame, peanut and tree nuts), and non-allergic controls showed a distinct microbial profile for FA to different foods characterized with an underrepresentation of *Prevotella copri* (65). In agreement, maternal carriage of *Prevotella copri* during pregnancy was also linked to a decreased risk of FA during infancy (66). Growing evidence supports a role for the gut microbiome in the pathogenesis and course of FA, with microbial dysbiosis preceding the development of FA (67). It has been reported that an elevated Enterobacteriaceae/Bacteroidaceae ratio in early infancy as well as lower microbial species richness in the infant (n=166, ages 3 and 12 months) might be a predictor of egg, milk, and peanut sensitization (determined by SPT) at age of 12 months, adjusting for birth delivery mode, antibiotic use, or breastfeeding (68). This raises the question of whether FA can be predicted using gut microbiome biomarkers (69). A study with 319 subjects enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) study showed that infants at risk of asthma exhibited transient gut microbial dysbiosis during the first 100 days of life characterized by lower relative abundance of *Lachnospira*, *Veillonella*, *Faecalibacterium*, and *Rothia* species (70). Another study reported lower relative abundance of *Citrobacter*, *Oscillospira*, *Lactococcus* and *Dorea* in stool samples collected at age 3-6 months in children who had FA (milk, egg, peanut, wheat, soy, or other nut allergy) by the age of 3 years (71). In addition, Firmicutes including Clostridia were enriched in the gut microbiota of infants at age 3-6 months whose milk allergy resolved at 8 years of age (72), suggesting a potential predictive role of gut microbiota composition for FA.

Interestingly, the specific microbiota signature may distinguish infants with IgE-mediated from non-IgE-mediated FA. Infants with IgE-mediated FA had increased levels of *Clostridium sensu stricto* and *Anaerobacter* and decreased levels of *Bacteroides* and *Clostridium* XVIII, with a positive correlation between *Clostridium sensu stricto* and serum sIgE (73). However, as with observational studies, it is not possible to assess causation between changes in microbial composition and FA (74). A study in adults with FA showed the opposite results with reduced Clostridiales, and increased Bacteroidales (75), suggesting that the changes observed in microbiota associated with allergy may be different depending on other factors such as age, ethnicity, geographical location and lifestyle.

It is widely known that early infant microbiota is influenced by several factors including mode of birth, antibiotic use and environmental exposures that can contribute to the dysbiosis linked to allergy development (**Figure 1)** and would provide opportunities to develop strategies aimed to microbial modulation and decrease risk of FA (76).

C-section delivery and antibiotic exposition. Available evidence indicates that C-section is a possible risk factor for FA as newborn infant bypasses microbial exposure, naturally happening during vaginal delivery, obtaining a distinct gut microbiota (77). In general, infants born by C-section have lower levels of *Bacteroides* and lower diversity which is a pattern also observed to precede the development of allergic symptoms in several studies (78). However, there is no clear evidence on C-section association with a higher risk of FA development, with studies producing contradictory results (79, 80). On the other hand, a 7-fold increased risk of parental-reported fish or nuts allergy and a 4-fold increased risk of confirmed egg allergy was reported (81) among high-risk children born via C-section. C-section was found to be associated with other allergic diseases, such as allergic rhinitis (OR: 1.23; 95% CI: 1.12, 1.35), asthma (OR: 1.18; 95% CI: 1.05, 1.32) and allergic sensitisation to foods (OR: 1.32; 95% CI: 1.12, 1.55) (82). Most of the C-sections are associated with

antibiotic intrapartum. Antibiotic use (particularly cephalosporins and sulfonamides) including its frequency during pregnancy and first year of life was linked with an increased risk of FA development (83), and is likely to reflect an indirect effect via infant gut microbiota dysbiosis (84, 85).

Breastfeeding practices. It has been shown that infants with CMA had an increased gut microbiota diversity and a higher prevalence of members belonging to the *Lachnospiraceae* family (Firmicutes phyla) as compared to non-allergic infants (86). However, another study showed an inverse association between the early gut microbial diversity and the risk of allergic sensitization (87). A low gut microbiota richness, overrepresentation of Enterobacteriaceae and underrepresentation of *Bacteroidaceae* (Bacteroidetes phyla) at 3 months of age were associated with food sensitization in a subset of the CHILD study (68). Those associations were found among infants who were vaginally delivered, exclusively breastfed and unexposed to antibiotics.

Breastfeeding practices were associated with lower diversity and higher levels of *Bifidobacterium breve* and *B. bifidum* (Actinobacteria phyla), and the cessation of breastfeeding resulted in faster maturation of the gut microbiota, as marked by an increase in the members belonging to Firmicutes phyla (88). However, formula-fed infants had more diverse microbiota with higher proportions of *Clostridium spp* (Firmicutes phyla), and Enterobacteriaceae members (Proteobacteria phyla), but with lower bacterial count (89). Recent studies have shown that breastmilk with a reduced microbial richness in the first month of life may play an important role in allergy development during childhood (90). Thus, the protection against allergy development provided by human milk may be attributable to the effect on the infant gut microbiota or direct effects on immune system; however, further studies are needed to evaluate the effect of breastfeeding and milk specific compounds on FA (91).

Environmental exposures. Associations between living in affluent countries and allergic disease development are well-known and FA is no exception to the rule. A higher socioeconomic status (92) or living in developed societies were associated with an increased risk of FA development, though it is possible that variations in frequencies of studies and methodological variation also contributes to these geographic variations (4). Researchers suggest that farming lifestyle exposes pregnant women and their offspring to a wide variety of microorganisms, which urban inhabitants lack. Data from two large, prospective, cohorts showed that exposure to a greater variety of environmental microorganisms was associated with a reduced risk of asthma development in PARSIFAL (OR: 0.62; 95% CI: 0.44, 0.89) and in GABRIELA (OR: 0.86; 95% CI: 0.75, 0.99) (93).

4. Dietary interventions

4.1. Macronutrients and micronutrients associations with FA

A recent systematic review was published recently, suggesting that supplementation with fish oil (a source of long chain omega-3 fatty acids) during pregnancy and lactation may reduce risk of allergic sensitisation to egg (RR: 0.69; 95% CI: 0.53, 0.90; I2 = 15%; Absolute Risk Reduction: 31 cases per 1,000; 95% CI: 10, 47) (94). The *Grading of Recommendations Assessment, Development and Evaluation* certainty of these findings was moderate. In addition, *in vitro* and *in vivo* studies have demonstrated that n-3 PUFA can modulate the activity of dendritic cells, T cells, and IgE production by B cells, reducing allergic sensitisation (95).

Although vitamin D deficiency was linked with the development of allergic diseases (96), data relevant for FA is limited. Vitamin D deficiency linked with GG genotype producing less vitamin D binding protein (DBP) was associated with a higher prevalence of egg and peanut allergy in 1- and 2-year-old infants (97). Use of vitamin D supplements during

pregnancy as a prevention of FA was, however, unsuccessful, both in a RCT (RR: 1.92; 95% CI: 0.57, 6.50) (98) and a case-control study (OR: 1.50; 95% CI: 0.78, 2.88) (99). Supplementation during the first year of life resulted in a reduced risk of FA development during the first 12 months of life (RR: 0.49, 95% CI: 0.27, 0.88) (99). However, the confidence in this estimate is also very low owing to indirectness of the evidence and risk of bias, as reported in a recent systematic review on the subject (100). Overall, there is currently not enough evidence to suggest that vitamin D supplements for pregnant and/or breastfeeding women or infants have an effect on FA development (39).

4.2. Dietary interventions targeting the microbiota modulation: pre- and probiotics

Targeted and personalized nutrition is an emerging strategy to approach FA in early infancy including microbiome-modifying interventions with probiotics (*Lactobacillus acidophilus* LAVRI A1, *Lacticaseibacillus rhamnosus* GG), prebiotics (long-chain fructo-oligosaccharides, short-chain galacto-oligosaccharides) and human milk oligosaccharides (2′-fucosyllactose, lacto-N-neotetraose) (101). The pathogenesis of FA in early infancy and other associated events such as dermatitis or asthma is still largely unknown, but increasing evidence suggest that they are associated with a perturbation of gut microbiome, namely microbial dysbiosis, leading to alterations in immune system which could influence the occurrence of FA (102). In addition, FA derives from a defect in immune tolerance mechanisms. Immune tolerance is modulated by gut microbiota composition and function. Therefore, the potential use of probiotics has been highlighted to counteract microbial dysbiosis linked to FA and boost microbial modulated tolerance because probiotics could interact with host microbiota and the host immune system at the same time (103). In infants, supplementation with specific probiotic strains may reduce the risk of sensitization to cow's milk (RR: 0.60; 95% CI: 0.37, 0.96) (104) although the quality of evidence is considered low. In general, those studies combined maternal and infant supplementation and it is unclear if

the effect is due the combination or the specific intervention (104-106). A systematic review and meta-analysis was published recently, suggesting that probiotics intake during late pregnancy and lactation may reduce risk of eczema (RR: 0.78; 95% CI: 0.68, 0.90; I^2: 61%; Absolute Risk Reduction: 44 cases per 1,000; 95% CI: 20, 64) (94). There are some studies associating the consumption of oligosaccharides in early life with reduced incidence of atopic dermatitis and other allergy manifestations (107, 108) with a lack of evidence in food allergy and human studies. However, the evidence on the use of prebiotics, probiotics and synbiotics in breastfeeding mothers and infants to reduce the risk of FA is inconclusive (39). In a randomized controlled trial, specialized infant formula enriched with fructo-oligosaccharides and *Bifidobacterium breve* M-16V was able to restore altered microbiota in non-IgE mediated cow´s milk allergic infants bringing it close to the healthy breastfed microbial profile when compared with the same formula without the synbiotic (109). Increasing evidence suggests that shifts in the neonatal gut microbiota composition, activity and diversity are implicated in the pathogenesis of FA (**Table 4**).

5. Evidence for the role of microbial metabolites in FA

Increasing data is showing the key role of metabolites in the host-microbe interaction as messengers and signals between microbiota and immune system with impact on human health. A comprehensive understanding of how microbiota-derived metabolites influence the human immune system and health is critical for the rational design of therapies for microbiota-driven diseases (110). Different dietary patterns change the proportions and type of microbial groups, influencing host exposure to microbial metabolites (111) which in turn produce epigenetic changes. Although no data is available for infants <1 year of life, in older children and adults, a balanced low-fat and high-fiber diet may be important in preventing perturbation of gut microbiome and preserving a functional immune system (112). Little is known about the role of microbial metabolites in FA but evidence is showing the impact of

diet including prebiotics on the production of microbial metabolites as SCFA, polyamines and even other compounds as toxins (LPS, staphylococcal enterotoxin B, etc.).

5.1. Short chain fatty acids

Metabolites produced by intestinal microbiota, and in particular SCFAs, play a critical role in mediating the effect of the gut microbiota on Treg proliferation and differentiation both *in vitro* and *in vivo* (113). The molecular mechanisms for this are not clearly elucidated but butyrate may suppress NF-κB and STAT1 activation and induce differentiation of colonic Treg cells by enhanced histone acetylation (113-116). Moreover, these effects are not confined to the gastrointestinal tract, and both butyrate and propionate have been reported to influence peripheral Treg development (117). The mechanisms involved in SCFA regulation of T-cell differentiation may include the control of cellular metabolism and the G-protein-coupled receptors signaling pathways (118), and involve strong epigenetic regulation through inhibition of histone deacetylases (102). In particular, the effect of butyrate on Treg differentiation may be through the increase of histone H3 acetylation in the *FOXP3* locus (117), and propionate seems to increase the expression of *FOXP3* and *Il10 (119)*. These results may explain the benefits of dietary fibers and bacteria, such as *Akkermansia municiphila*, *Faecalibacterium prausnitzii, Eubacterium, Bifidobacterium, Clostridium and Ruminococcus*, typical SCFAs producers, that can increase colonic luminal SCFA concentrations and modulate immune system response (120, 121).

Some specific SCFA have been reported to influence FA. In details, butyrate has a well-known inhibitory effect on histone deacetylases (114) and can induce the expression of non-coding RNAs (113, 116). Furthermore, a lower butyrate production and shifted gut microbiota composition towards an enrichment of *Bacteroides* and *Alistipes* genus have been reported in infants with non-IgE-mediated CMA (122). Low levels of SCFAs at one year of age has been associated with questionnaire-reported symptoms of FA at four years (123). In

addition, propionate has been associated to an increased expression of FOXP3 and IL-10 in colonic Treg cells (119). There are signals of an association between SCFA and Treg cells development and function by epigenetic mechanisms, but the influence of this association in the risk of FA is still not clear.

5.2. Other microbial metabolites

It has been suggested that some other microbial metabolites such as Staphylococcal enterotoxin B could act as adjuvants of food allergens during simultaneous exposure via skin (74). *Staphylococcus aureus* colonization of the skin has been associated with FA to peanut, egg white, and cow's milk in patients with atopic dermatitis, and may be associated with skin barrier dysfunction and immune system dysregulation (124). Bacterial LPS are strong immunostimulants that can induce tolerance at certain doses (125). Their role in allergy seems to be conditioned by the timing of exposure, the presence of preexisting disease, and polymorphisms in the genes that encode endotoxin receptors (126). Evidence in humans is not clear but results from animal studies indicate LPS might prevent adverse IgE-mediated reactions by regulation of Th2-type responses (127) and suppression of mast-cell responses (128).

There is substantial evidence that intestinal bacteria can produce significant amounts of folate as well as other B-vitamins complementing the dietary intake (129), including generally recognize beneficial microorganisms such as bifidobacterias and lactic acid bacteria (115). These B-vitamins, and particularly folate, play a crucial role in epigenetic regulation as donors of methyl groups for DNA, RNA and protein methylation (130, 131). Folate-induced changes in DNA methylation may modify gene expression in T-helper cells (132), which has been proposed as a plausible mechanism underlying associations between folate and several diseases such as asthma (129), child wheeze (133) and allergy (134). For FA, it is still largely underexplored with contradictory results depending on the studies (132). Most of the few

studies conducted to date suggest that maternal folate exposure is not associated with the development of FA (132). However, a retrospective study suggested that maternal folic acid supplementation in dosages higher that recommended may be a risk factor for allergy development (135). Emerging evidence on the role of biotinylation upon immune function (136-138) and microbial metabolites such as polyamines (139-143) indicate potential further links between the gut microbiome and allergy by epigenetic regulation of genes modulating the activity of T and B lymphocytes, and proinflammatory cytokine expression (111, 136-144).

6. Recommendation / Guidance for future research

FA research is now experiencing an exciting new era thanks to the advances on immunological, microbiological, and epigenetic factors and their integration allowing an increased knowledge on risk factors and potential biomarkers. However, limited data is available to identify potential biomarker or biomarkers combination determining a risk reduction in FA. The EAACI has recently published a systematic review as a source of evidence to support the development of FA prevention guidelines (39). This systematic review included forty- six intervention studies to reduce the risk of FA in infancy (up to 1 year) or early childhood. Different interventions during pregnancy, lactation and infancy including dietary avoidance of food allergens, vitamin supplements, fish oil, probiotics, prebiotics, synbiotics, and emollients were included. Results showed that interventions have little or no effect on preventing FA, but the evidence is very uncertain. The systematic review concluded that most of the evidence has been published in the last 10 years, and still no clear data are available on preventing FA. There is a need to validate the potential benefits of early introduction of food allergens in a wider range of populations. Furthermore, there is a lack of studies analyzing serial and longitudinal biomarkers from birth up to adulthood, and clear biomarkers have not been identified until now. Promising potential biomarkers associated

with FA such as the depletion of key microbial components (e.g. *Bifidobacterium* and Bacteroides) or methylation profiles in the *FOXP3* and *IL-10* genes should be deeply evaluated in future studies.

To bridge the gap, more data is required on the maternal impact during gestation upon fetal immune regulation as well as the immunometabolic profile of breastmilk composition (immune cells, cytokines, hormones). There are also a limited number of studies focusing on immunology, microbiome, diet and few assess across the board. More cohort and intervention studies are needed to confirm which methylation profiles are suitable as biomarkers to monitor risk reduction of FA. Thus, designing nutritional intervention trials aimed at risk reduction of FA, or induction of tolerance may need stratification based on specific risk factors to be able to come to a design that is still feasible to execute. Indeed, low incidence of oral food challenge confirmed FA in the general population requires high numbers of infants to be able to detect a significant effect of an intervention. This review of currently available and emerging biomarkers linked to allergy can inform the design of future intervention studies. The available literature suggests that a highly collaborative approach spanning nutritional, genetic and microbial biomarkers will be valuable in identifing panels of biomarkers which best predict food allergy, its severity or its remission.

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Table 1. Summary of the most common and specific determinants impacting microbiota and risk to develop food allergy.

	Factors associated with higher risk of food allergy	**Factors associated with lower risk of food allergy**	**Factors with no association with higher/lower risk of food allergy**
Factors increasing microbial dysbiosis	Antibiotic use during pregnancy and first year of life	-	Formula feeding
	Cesarean delivery		Low fiber/high fat diet
	Exposure to bacterial enterotoxins		
	Vitamin D deficiency		
Factors improving microbial equilibrium	-	Farm/rural lifestyle	Outdoor activities
		Pet exposure in early life	Breastfeeding
		Having older siblings	Probiotics/fermented products
		Exposure to an increased diversity of foods in early life	Less processed food
		Ingestion or aryl hydrocarbon receptor ligands (cruciferous vegetables)	
		N-3 polyunsaturated fatty acids	
Factors with no proven impact on microbial dysbiosis/equilibrium	Early cutaneous exposure to food allergens in the environment	Early oral exposure to foods	-
	Family history of allergic disease		
	Prior diagnosis of atopic disease like eczema		
	Higher socioeconomic status		
	Living in developed societies		

Table 2 - Genetic loci associated with food allergy, their potential link with FA and evidence supporting their use as a biomarker.

Name	Genetic risk factor	Role	Potential link with FA	Reported utility as biomarker?	References
Toll-like receptor 6	TLR6	Pathogen recognition and activation of innate immunity	TLR function can be altered by early environmental and microbial exposures	Generally associated with allergic sensitization	(15, 145)
EMSY transcriptional repressor	C11orf30	Repressor of BRCA2 protein	Involved in epigenetic regulation of gene expression	Identified as genetic risk factor for peanut allergy and food allergy	(15, 146)
Signal transducer and activator of transcription 6	STAT6	Central role in IL4 mediated responses	Polymorphisms have been associated with age of tolerance induction.	Age of tolerance development for cow's milk was significantly higher in children with the GG genotype at rs324015 of the STAT6 gene compared with those with the AA+AG genotype (2 years [range, 1.5-3.9 years] vs 1.2 years [range, 1.0-2.2 years]) (P = .02)	(15, 147)
Solute carrier family 25 member 46	SLC25A46	Promotes mitochondrial fission and prevents the formation of hyperfilamentous mitochondria	Involved in the association between food allergy and atopic dermatitis	Polymorphism SLC25A46 was associated with higher risk of food allergy	(15, 148)

Major histocompatibility complex, class II, DQ beta 1	HLA-DQB1	Plays a central role in the immune system by presenting peptides derived from extracellular proteins.	Peanut allergic specific loci in the human leukocyte antigen (HLA)-DQ and –DR region were found in a large cohort study.	Several polymorphisms associated with peanut, milk and egg allergy	(15, 16, 149)
Interleukin 1 receptor like 1	IL1RL1	Involved in the function of helper T cells	ST2, β-chain of IL-33 receptor	Generally associated with allergic sensitization	(15)
LIM domain containing preferred translocation partner in lipoma	LPP	Involved in cell-cell adhesion and cell motility. This protein also shuttles through the nucleus and may function as a transcriptional co-activator.	Allergic sensitization	Generally associated with allergic sensitization	(15)
MYC proto-oncogene, bHLH transcription factor	MYC	Plays a role in cell cycle progression, apoptosis and cellular transformation.	Down-regulated among children with food allergy	Generally associated with allergic sensitization and food allergy	(15, 150)
Interleukin 2	IL2	Proliferation of T and B lymphocytes	Allergic sensitization	Generally associated with allergic sensitization	(15)
Major histocompatibility complex, class I, B	HLA-B	Central role in the immune system by presenting peptides derived from the endoplasmic reticulum lumen	Allergic sensitization	Generally associated with allergic sensitization	(15)
Filaggrin	FLG	Role in skin barrier function	Indirect association with food allergy	Filaggrin loss-of function mutations are associated with food allergy in older children via eczema and food allergen	(20, 21, 23, 151)

				sensitization in their early childhood	
Interleukin 13	IL13	Involved in several stages of B-cell maturation and differentiation	IL13 polymorphism rs1295686 (in complete linkage disequilibrium with functional variant rs20541) is associated with challenge-proven food allergy.	IL-13 gene polymorphisms have also been identified as biomarkers of IgE-mediated food allergy and are a predictor of cord blood IgE concentrations	(152)
Catenin alpha 3	CTNNA3	Cell-cell adhesion	Knockdown of CTNNA3 resulted in upregulation of CD63 and CD203c in mononuclear cells upon PMA stimulation	Copy number variation impacting CTNNA3 has been associated with pediatric food allergy	(153)
RNA binding fox-1 homolog 1	RBFOX1	Regulates alternative splicing events	Association with food allergy at a genome-wide scale	Generally associated with pediatric food allergy	(153)
GC vitamin D binding protein	GC/DBP	Binds to vitamin D and its plasma metabolites and transports them to target tissues	GG genotype produces less vitamin D binding protein (DBP)	Vitamin D deficiency linked with GG genotype producing less vitamin D binding protein (DBP) was associated with a higher prevalence of egg and peanut allergy in 1 and 2 year-old infants.	(154)
Indoleamine 2,3-dioxygenase 1	IDO1	Modulates T-cell behavior	High IDO activity is associated with non-responsiveness to food allergens despite	Associated with tolerance to food allergens	(155)

			allergen sensitization.		
Sirtuin 1	SIRT1	Functions of human sirtuins have not yet been determined	Negatively regulates FcεRI-stimulated mast cell activation and anaphylaxis	Generally associated with anti-allergic response	(156, 157)

Table 3 – Epigenetic changes associated with food allergy.

Study	**Where identified**	**Main findings**	**Potential mechanism of action**	**Reported utility as biomarker?**	**References**
DNA methylation profiles (~450,000 CpGs) of peripheral immune cells (CD4+ T-cells)	Children with IgE-mediated food allergy	179 differentially methylated sites of loci associated with the disease phenotype, and 96 CpG sites DNA methylation profile discriminated food allergic vs healthy infant	MAP kinase pathway → Dysregulation of DNA methylation at MAPK signaling-associated genes during early CD4+ T-cell development may contribute to suboptimal T-lymphocyte responses in early childhood associated with the development of food allergy	Predicted clinical outcomes with an accuracy of almost 80%. MAP kinase pathway was most prominently associated with CpGs that were predictive of food challenge	(158, 159)
DNA methylation profiles	Egg allergy	DNA methylation profiles of T cells discriminate infants with persistent egg allergy compared with those who had outgrown egg allergy.	Methylation of metabolic (RPTOR, PIK3D, MAPK1, FOXO1) and inflammatory genes (IL1R, IL18RAP, CD82) affected	Data about predictive potential not available	(150)

DNA methylation profiles	Cow's milk allergy	Cow's milk allergic infants showed hypermethylation in whole blood compared to controls and tolerant group	Differential methylation patterns on DHX58 (innate immune response), ZNF281 (transcriptional regulation), EIF42A (interferon pathway) and HTRA2 (smooth muscle contraction) between groups	Data about predictive potential not available	(160)
DNA methylation profiles and single-nucleotide polymorphisms (SNPs)	Peanut allergy	DNA methylation of the HLA-DQB1 and HLA-DRB1, IL4, IL12B, IL2, brain-derived neurotrophic factor (BDNF), IL17F, CXCL12, CCR7, runt-related transcription factor 1 (RUNX1), CD3ε, and SERPINE1 IL1B and IL6 has been associated with peanut allergy	Increased protein secretion in response to allergen-specific stimulation. Additional functional studies are needed.	DNA methylation signature combinations may have superior diagnostic potential than serum peanut–specific IgE	(16)
Th1-Th2	Cow's milk allergy	DNA methylation profiles differs with cows milk allergy	DNA methylation profiles of IL-4, IL-5, IL-10 and IFN gamma genes between infants with active cows milk allergy and those who outgrew their cows milk allergy.	GATA3 in Th2 cells *Ex vivo* PBMC cytokine profile in predicting cows milk allergy: TNF, IL10, IL12 higher in cows milk allergy patients compared to controls	(161-163)

Th1-Th2	Cow's milk allergy	DNA methylation of FOXP3, Th1/Th2 cytokine genes in IgE-mediated in children with cow's milk allergy treated with an extensively hydrolysed formula including a probiotic (test formula) versus a control formula	FOXP3, IL-10 and IFNg demethylation rate was higher and IL-4 and IL-5 demethylation rate was lower in the test formula group	Intervention promotes regulatory and immune suppressive immune factors and at the same time decrease activity of Th2 type genes.	(164)
FOXP3	Peanut allergic infants & cow's milk allergic infants	immune tolerant participants had ↑ai-Treg with greater suppressive function, and with ↑ FOXP3 hypomethylation	Oral immunotherapy in peanut allergic infants increased antigen induced regulatory T cell function and hypomethylation of FOXP3 in infants that became tolerant	Data about predictive potential not available	(165)
	Cow's milk allergy	↓FOXP3 gene demethylation in children with active IgE-mediated cows milk allergy	Formula selection influenced the FOXP3 T-cell specific demethylation region demethylation profile	Data about predictive potential not available	(166)
Methylation levels taken from mononuclear blood cells at 405,658 CpG islands across the genome (machine learning approach)	40 samples for training, 10 samples for cross-validation, and 8 completely hidden samples for testing	Novel 13-gene signature to diagnose clinical reactivity: chr1p13 (SARS), chr7p22 (MAFK), chr11q14 (PANX1), chr9p22 (SLC24A2), chr8p21 (KIF13B), chr10q26 (CTBP2), chr10q11 (ARID5B), and chr10q23 (FAM190B).	These genes are mapped to several canonical Wnt pathways, GO, and positional gene sets with functional association with the immune system	The 18-CpG signature mapped to 13 genes is a strong biomarker of FA with a 94-96% accuracy	(167)

Table 4 – Perinatal Probiotics, Prebiotics and Synbiotics for cow's milk allergy management and allergy prevention: summary of clinical studies and meta-analyses. CMA: cow's milk allergy; RT: randomized trials.

Clinical studies						
Strain(s)	**No. subject**	**Intervention Time**	**Target**	**Outcome(s)**	**Study type**	**Reference**
Lactobacillus LGG $1x10^9$ CFU	100 infants diagnosed with CMA	4 weeks	Management of CMA	Significant improvement in symptoms of infants diagnosed with CMA No impact on abdominal pain, constipation and dermatitis	Randomised double-blind placebo controlled study	(168)
Synbiotic formula with a combination of *B. breve* M-16V and chicory-derived neutral oligofructose, long-chain inulin	122 infants Synbiotic n= 35 Control n= 36 Reference n= 51	8 weeks	Management of severe or complex non-IgE mediated CMA	↑ percentage of *Bifidobacterium* and lower % of *Eubacterium rectale*/*Clostridium coccoides* group in the test group No significant results for the faecal secretory IgA and short chain fatty acids	Double-blind, randomised clinical trial with non-randomised breastfed reference group	(169)
Lactobacillus rhamnosus and *Bifidobacterium animalis* subsp *lactis*	290 participants approx 10 month of age N= 144 probiotic N= 146 placebo	6 months	Allergic diseases and sensitization.	↓ incidence of eczema No effect on the incidence of asthma and conjunctivitis or sensitization	Randomised double-blind, placebo-controlled intervention	(170)

Amino-acid-based formula (AAF) with fructo-oligosaccharides and *Bifidobacterium breve* M-16V.	51 infants aged <13 months N= 35 (test) N= 36 (control)	Infant intervention for 8 weeks	Management of infants with suspected/proven CMA.	↑bifidobacteria in the AAF with prebiotic and probiotic	Randomised double-blind, placebo-controlled intervention	(109)
L. rhamnosus GG; *L rhamnosus* LC705 (DSM 7061), *Bifidobacterium breve* Bb99 (DSM 13692), and *Propionibacterium freudenreichii* ssp. *shermanii* JS (DSM 7076).	891 mothers with infants at high risk for allergy Probiotic n = 445 Placebo n = 446	Maternal-infant intervention Follow-up until 5 years	Allergy prevention	↓ IgE-associated allergic disease occurred in cesarean-delivered children No allergy-preventive effect that extended to age 5 years	Randomised Double-blinded, placebo-controlled study	(171)
Meta-analyses						
Strain(s)	**No. subject**	**Intervention Time**	**Target**	**Outcome(s)**	**Study type**	**Reference**
Different strains	10 RT (n = 845; probiotics, 422; control, 423) Infants	Different intervention times	Management of infants with suspected/proven CMA.	No impact on hematochezia In confirmed CMA, probiotics ↑ acquisition of tolerance to CMP at the end of 3 years	Meta-analysis	(172)
Single or multiple organisms, given as capsules, powder, or part of a drink or infant formula milk	28 trials RT 6,705 participants	Maternal-infant intervention	Allergy prevention	↓ risk of eczema and/ or atopic eczema at age ≤4 years. ↓ allergic sensitisation to cow's milk at age 1 to 2 years	Systematic review and meta-analysis	(94)

Combinations of lactobacilli and bifidobacteria	17 trials 2947 infants	Maternal-infant intervention	Allergy prevention	↓ risk of atopic eczema ↓ risk of food hypersensitivity When probiotics were administered either only prenatally or only postnatally, no effects on atopy and food hypersensitivity	Systematic review and Meta-analysis	(173)
Combinations of lactobacilli and bifidobacteria	17 RT 4755 children Probiotic n= 2381 Control n= 2374	Maternal intervention during pregnancy	Allergy prevention	↓ risk ratio for eczema No impact on asthma, wheezing or rhinoconjunctivitis	Meta-analysis	(174)

Figure 1

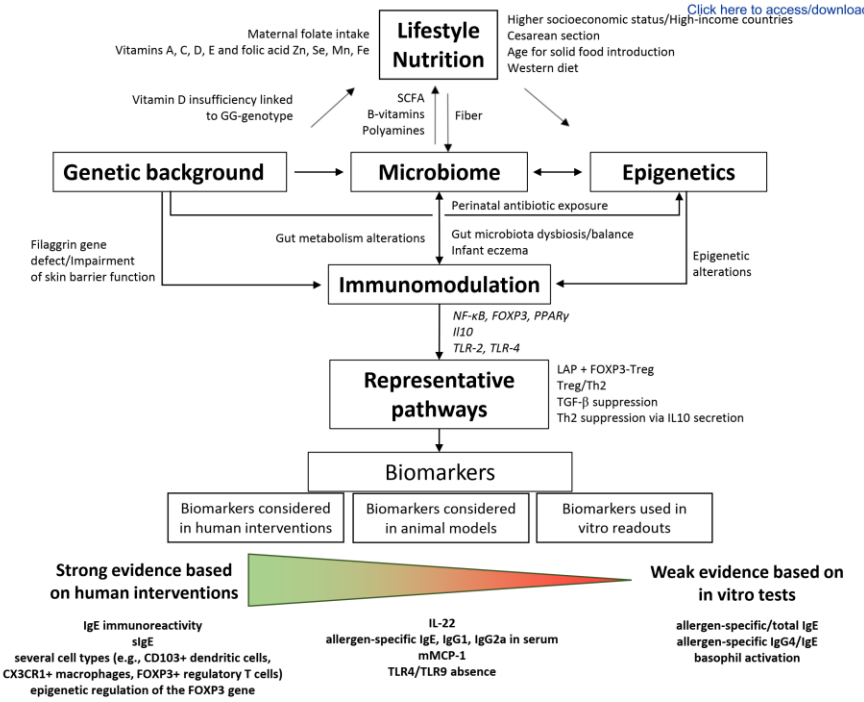

Figure 1 - Level of evidence of different biomarkers and interactions between genetic background, lifestyle, and epigenetics factors on the interplay between microbiota and the immune system on food allergy. CD103: integrin αEβ7; CX3CR1: C-X3-C Motif Chemokine Receptor 1; FOXP3: Forkhead box P3; LAP: Latency associated peptide; mMCP-1: Mouse mast cell protease-1; PPAR: peroxisome proliferator-activated receptors; TGF-β: Transforming growth factor beta; TLR: Toll-like receptor; Treg: T-regulatory.