

The Implications of DNA Methylation on Food Allergy

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Abstract

Food allergy is a major clinical and public health concern worldwide. The risk factors are well defined, however, the mechanisms by which they affect immune development remain largely unknown, and unfortunately the effective treatment or prevention of food allergy is still being researched. Recent studies show that the genes that are critical for the development of food allergy are regulated through DNA methylation. Environmental factors can affect host DNA methylation status and subsequently predispose people to food allergy. DNA methylation is therefore an important mediator of gene-environment interactions in food allergy and key to understanding the mechanisms underlying the allergic development. Indeed, the modification and identification of the methylation levels of specific genetic loci have gained increasing attention for therapeutic and diagnostic application in combating food allergy. In this review, we summarize and discuss the recent developments of DNA methylation in food allergy, including the patho-

genesis, therapy, and diagnosis. This review will also summarize and discuss the environmental factors that affect DNA methylation levels in food allergy.

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Introduction

The prevalence of food allergies has been increasing rapidly worldwide, particularly in children [1]. For example, in the USA, around 8% of children are reported to have food allergy [2]. In Europe, the lifetime prevalence and point prevalence for self-reported food allergy are around 17 and 6%, respectively [3, 4]. An Australian cross-sectional population study found that 10% of children aged 1–5 years developed an allergy to foods [5]. Food allergies are regarded as a new wave of allergy epidemic because of their increasing prevalence, their potentially fatal implications, and their vast medical and economic impacts [6]. The most common food-causing allergies include peanut, milk, and hen's egg [7]. Peanut allergy is potentially a lifelong disease [7], unlike cow's milk and hen's egg allergy [8], and may cause life-threatening reactions. The risk factors are well defined, how-

ever, the mechanism by which they affect immune development remains largely unknown for food allergy, and unfortunately the effective treatment or prevention for food allergy is still being researched [7]. Among all of the current treatments, immunotherapy has shown great promise by desensitizing patients with food allergies [9]. However, larger, well-designed studies are needed to fully evaluate the effects of food immunotherapy.

Nowadays there is an increasing recognition that epigenetic disruptions play an important role in the pathogenesis of diseases. Epigenetic disruptions regulate genomic adaption to environmental factors, and can thus initiate disease development [10]. Among all of the epigenetic disruptions, DNA methylation is the most commonly known. DNA methylation occurs by the addition of a methyl (CH₃) from S-adenosyl methionine to the DNA strand itself, often to the 5-carbon of a cytosine ring that resides at a CpG site [11–13]. By doing this, DNA methylation modifies the function of the DNA, leading to changes in gene expression. Moreover, DNA methylation can render the chromatin, or protein structure surrounding the DNA molecule, into an “inactive” state, leading to gene silencing [14].

In this review, we report on the recent developments in epigenetic studies of food allergy, with a specific focus on DNA methylation.

DNA Methylation in the Pathogenesis of Food Allergy

To date, the majority of epigenetic studies have focused on asthma [15–18]. Nonetheless, disruptions in DNA methylation levels may also play a critical role in the development of allergic diseases [19]. DNA methylation levels can affect the development of allergic reactions to different types of foods (Table 1).

Interleukins

The interleukins are a group of cytokines, which are biologically active protein mediators [20] that play a crucial role in immune responses and are related to food allergy. Song et al. [21] conducted an animal (mouse) study in order to examine the relationship of allergy susceptibility and Th2-biased epigenetic alterations. The offspring of mothers with peanut allergy and the offspring of naive mothers were both sensitized with peanut. Blood sera were collected weekly during sensitization and 1 day be-

fore challenge by means of submandibular venepuncture to monitor the antibody response to peanut sensitization. It was found that the DNA methylation of *IL-4* in the offspring of mothers with peanut allergy was lower than in the offspring of naive mothers. The study also found that *IL-4* methylation levels showed a negative correlation with IgE production. This demethylation of *IL-4* was suggested to impair Th2 responses and IgE class switching, resulting in the development of food allergy.

In another study investigating the underlying mechanisms for the development of food allergy, Berni Canani et al. [22] assessed the DNA methylation of CpGs in the promoter regions of genes from peripheral blood mononuclear cells as well as the serum levels of IL-4, IL-5, IL-10, and IFN- γ in 40 children. These children were divided into 3 groups: children with active IgE-mediated cow's milk allergy (group 1), children who acquired tolerance to cow's milk proteins (group 2), and healthy children (group 3). A venous blood sample (4 mL) was obtained from these children for epigenetic analysis. This study showed that the DNA methylation of *IL-4* and *IL-5* in patients with cow's milk allergy was lower, and the DNA methylation of *IL-10* and IFN- γ was higher, compared to the healthy controls. Moreover, this study also found a significant and negative correlation between methylation levels of the cytokine promoter regions and the respective serum concentrations. The data revealed that demethylation of *IL-4* and *IL-5* and increased methylation of *IL-10* and IFN- γ played an important role in the pathogenesis of food allergy.

Other than the above interleukins, IL-17A is also found to be associated with the development of food allergy and is suggested to be one of the key mediators in regulating inflammatory processes [23]. The concentrations of IL-17A were quantified and compared between 30 patients with food allergy and 10 healthy subjects. The patients with food allergy had higher levels of IL-17A than the healthy controls. The mechanism by which IL-17A affects the pathogenesis of food allergy remains unknown. However, it was suggested that the *IL-17A* gene was regulated by promoter methylation, which affects pro- and anti-inflammatory responses of the immune system leading to allergy development [24]. Further studies are required to confirm the relationship between methylation of *IL-17A* and the development of food allergy.

HLA-DR and HLA-DQ Gene Regions

The major histocompatibility complex (MHC) class II system mediates the cell-mediated adaptive immune response [25–27]. There are 3 pairs of MHC class II α - and

Table 1. Genes associated with the immune system regulated by DNA methylation in food allergy

Genes	Cell type	Function	Type of allergy	Potential role	Reference
<i>IL-4</i>	CD4+ (Th2)	Enhance the differentiation of naive CD4+ T cells into IL-4-producing Th2 cells	Cow's milk	Demethylation of <i>IL-4</i> impairs Th2 responses and IgE class switching	21, 22
<i>IL-5</i>	CD4+ (Th2)	Regulate eosinophilic inflammation	Cow's milk	Demethylation of <i>IL-5</i> increases the risk of developing food allergy	22
<i>IL-10</i>	CD4+ (Th2)	Suppress allergic inflammation	Cow's milk	Increased methylation of <i>IL-10</i> induces oral tolerance	22
<i>IFN-γ</i>	CD4+ (Th1/2)	Suppress Th2 responses of allergic diseases	Hen's egg, cow's milk, peanut	Increased methylation of <i>IFN-γ</i> is associated with food allergy	22
<i>IL-17A</i>	CD4+	Increase allergic inflammation	Food allergy	Methylation of <i>IL-17A</i> is associated with food allergy	23
<i>HLA-DR</i> and <i>HLA-DQ</i>	CD4+ T cells	Present antigen-derived peptides, mostly of exogenous origin, to CD4+ helper T cells	Peanut	Recognize allergenic proteins, including peanut proteins, which then lead to specific IgE responses	28–31
<i>FOXP3</i>	T cells	Maintain immune tolerance to allergens	Peanut, cow's milk	<i>FOXP3</i> DNA methylation level is associated with food allergy	32, 33
<i>DHX58</i>	CD8+ and CD4+ T cells, natural killer cells, B cells, monocytes, granulocytes	Antigen recognition in the innate immune response to various RNA viruses and some DNA viruses	Cow's milk	Demethylation of <i>DHX58</i> is associated with allergic inflammation	37
<i>ZNF281</i>	CD8+ and CD4+ T cells, natural killer cells, B cells, monocytes, granulocytes	Repress GAST, which stimulates the stomach mucosa, produces digestive enzymes, ensures smooth muscle contraction, and releases histamines	Cow's milk	Increased methylation of <i>ZNF281</i> is associated with the gastrointestinal reactions	37
<i>EIF4E2</i>	CD8+ and CD4+ T cells, natural killer cells, B cells, monocytes, granulocytes	Stimulate postnatal growth	Cow's milk	Demethylation of <i>EIF4E2</i> is associated with allergic inflammation	37
<i>HTRA2</i>	CD8+ and CD4+ T cells, natural killer cells, B cells, monocytes, granulocytes	Regulate the perception of abdominal pain and smooth muscle contraction in the gastrointestinal tract	Cow's milk	Increased methylation of <i>HTRA2</i> is associated with gastrointestinal symptoms	37
<i>NDFIP2</i>		Associate with the immune system	Cow's milk	Demethylation of <i>NDFIP2</i> is associated with food allergy	38
<i>EVL</i>		Associate with the immune system	Cow's milk	Demethylation of <i>EVL</i> is associated with food allergy	38
<i>TRAPPC9</i>		Associate with the immune system	Cow's milk	Demethylation of <i>TRAPPC9</i> is associated with food allergy	38

β -chain genes, called the human leukocyte antigen (HLA) system, which includes *HLA-DR*, *HLA-DP*, and *HLA-DQ*. *HLA-DR* is the most prominent gene and more than 500 different *HLA-DR* protein sequences have been identified to date. *HLA-DQ* is closely linked to *HLA-DR*. The diversity of *HLA-DR/DQ* alleles signifies a versatile immune system of the host that can respond to a variety of pathogens, both at the level of the individual and at the level of the population [28]. DNA methylation affects

HLA-DQ by silencing the *HLA-DQ* region CCCTC transcription factor-binding insulators that separate the MHC class II subregions [29].

The first genome-wide association study of well-defined food allergy was conducted in Chicago [30]. This study recruited 2,759 participants who were sensitized to peanut. Of these 1,315 were children and 1,444 were parents. This study revealed that ethnic background was associated with DNA methylation of the *HLA-DQB1* and

HLA-DRB1 genes, and a linkage was found between DNA methylation and single-nucleotide polymorphisms (SNPs). It was revealed that 2 SNPs, namely rs7192 and rs9275596, influenced the DNA methylation pattern. DNA methylation sites that were specific for peanut allergy were identified in the *HLA-DR* and *HLA-DQ* gene region at 6p21.32, tagged by the SNPs rs7192 and rs9275596, in 2,197 participants of European ancestry. Proteins coded by *HLA-DQB1* and *HLA-DRB1* serve to present processed peptide antigens to CD4+ T lymphocytes, which in turn can stimulate the initiation of an antigen-specific immune response.

An epigenome-wide association study also confirmed the importance of *HLA-DQB1* in the development of allergic disease. Twelve children with IgE-mediated food allergy (hen's egg, cow's milk, and peanut) and 12 healthy children were recruited. Genome-wide DNA methylation profiles (approx. 450 000 CpGs) from CD4+ T cells were measured using the Illumina Infinium Human Methylation 450 Bead Array platform. The study showed that the DNA methylation status in specific regions of *HLA* may vary in different food allergies. Such change determines which antigen-derived peptides are presented to the T cells and potentially results in the dysregulation of food tolerance [31].

FOXP3

The regulatory T cell (Treg) is crucial in maintaining immune tolerance to allergens. The development and function of Tregs depends on the transcription factor forkhead box (*FOXP3*). The expression of *FOXP3* is regulated by the DNA methylation status of a methylation-sensitive enhancer (TSDR, Treg cell-specific demethylation region) [32], which is an alternative mechanism of disruption of the immune balance, causing host intolerance to exogenous substances.

A case control study investigated the function of Treg cells by investigating whether they were associated with immune tolerance in oral immunotherapy for peanut allergy [32]. Twenty-three cases with peanut allergy were compared with 20 age-matched allergic control subjects. The participants were required to undertake oral immunotherapy to peanut protein over the course of 24 months. Subsequently, the therapy was withdrawn for 3 months and followed by an oral food challenge at 27 months. The data revealed that the *FOXP3* DNA methylation level of patients with peanut sensitization was higher than that of the control participants. Moreover, a further 24-month follow-up study showed that the *FOXP3* DNA methylation level in patients who remained without allergic reac-

tions was decreased, compared with patients who resensitized to peanut [32]. Therefore, increased methylation of *FOXP3* is likely to play a crucial role in the development of peanut allergy.

An Italian cohort study also confirmed the importance of *FOXP3* in the development of food allergy and that its expression is regulated by DNA methylation [33]. Forty children aged 3–18 months were divided into 4 groups: 10 children with active IgE-mediated cow's milk allergy, 10 children who outgrew cow's milk allergy after dietary treatment with an extensively hydrolyzed casein formula containing the probiotic *Lactobacillus rhamnosus* GG, 10 children who outgrew cow's milk allergy after treatment with other formulas, and 10 healthy controls. The *FOXP3* TSDR demethylation and expression were measured in mononuclear cells that were purified from peripheral blood. This study showed that *FOXP3* TSDR demethylation was significantly lower in children with active IgE-mediated cow's milk allergy compared to either children who outgrew cow's milk allergy or the healthy children. This study further revealed that an extensively hydrolyzed casein formula containing the probiotic *L. rhamnosus* GG increased *FOXP3* TSDR demethylation, resulting in a tolerance reaction. Thus, the *FOXP3* methylation level appears to be important in cow's milk allergy and this can be influenced by the formula selection.

Filaggrin

The presence of mutations in *filaggrin* is the most frequently noted genetic factor for damage in the anatomical and functional barrier of the epidermis [34]. Mutations in the *filaggrin*-coding gene are linked to the occurrence of a constitutional reaction to protein antigens in food. Since the *filaggrin* mutation is often identified with atopic dermatitis, which usually coexists with food allergy, the independent effect of *filaggrin* on food allergy remains to be determined.

A recent study investigated the association of *filaggrin* and food allergy [35]. A total of 1,456 children were recruited and a skin prick tests (SPTs) were used to identify food allergen sensitizations. The results showed no direct effect of *filaggrin* mutations on food allergy at any age. However, there was a significantly indirect effect of *filaggrin* mutations on the risk of food allergy through eczema later in childhood, between the ages of 10 and 18 years. The barrier defect due to *filaggrin* deficiency makes the study subjects susceptible to the development of cutaneous sensitization through antigen-presenting cells and systemic atopic response [35]. Peanut sensitization can occur through the topical application of peanut oil to the

skin and cutaneous exposure through the presence of peanut allergen in the environment, and this process might be enhanced by a deficient barrier caused by *filaggrin* mutations or eczema [35]. Nevertheless, the importance of methylation at the CpG sites in the regulation of *filaggrin* expression in buccal cells is debated [36]. The study performed by Tan et al. [36] did not indicate a relationship between the methylation of the *filaggrin* gene promoter and allergy diseases.

Other Genes

Other than the abovementioned genes, differences in *DHX58*, *ZNF281*, *EIF42A*, and *HTRA2* gene DNA methylation are suggested to be associated with the development of cow's milk allergy as observed in the Dutch EuroPrevall birth cohort study [37]. In this study, Bisulfite converted DNA from the blood samples of 20 children with cow's milk allergy, 23 healthy controls, and 10 boys who outgrew food allergy. DNA was analyzed using the 450K Infinium DNA Methylation array [37]. This study revealed that increased methylation of *ZNF281* and *HTRA2*, and demethylation of *EIF4E2* and *DHX58* caused an allergic reaction. The increased methylation of *ZNF281* and *HTRA2* was suggested to be related to gastrointestinal symptoms while the demethylation of *EIF4E* and *DHX58* could lead to allergic inflammation.

Besides, an epigenome-wide association study of children from the USA revealed that the demethylation of the *NDFIP2*, *EVL*, and *TRAPPC9* genes was associated with the development of cow's milk allergy [38]. This study was conducted in 2 stages, the discovery stage and the replication stage. In the discovery stage, whole blood samples were collected from 106 Caucasian children with cow's milk allergy and 76 nonallergic and nonatopic Caucasian children. DNA methylation was measured at 485,512 genomic loci using the Illumina Human Methylation 450 arrays. In the replication stage, the top significant DNA methylation loci were validated using childhood whole blood samples from 25 Caucasian children from the Chicago Food Allergy Study and cord blood samples from 140 Afro-American children from the Boston Birth Cohort. This study found that the DNA methylation of the *NDFIP2*, *EVL*, and *TRAPPC9* genes was lower in children with cow's milk allergy compared to healthy children. These 3 candidate genes were suggested to be relevant for the immune system. The exact role of these annotated genes in cow's milk allergy remains to be determined. Altered DNA methylation is ob-

served in a number of different genes, implying that multiple cell pathways are required for allergic disease development.

DNA Methylation and Allergic Tolerance

The significance of DNA methylation for cellular regulation potentially provides a new strategy of allergy intervention and treatment. If DNA methylation can be artificially modified, it can change the corresponding gene and protein expression to prevent an allergic reaction or reverse allergic sensitization.

Tolerance can be induced through alterations in DNA methylation status of dendritic and CD4+ cells [32, 39–42]. The function of dendritic cells is to either induce productive immunity or maintain the state of tolerance to self-antigens and allergens. These dendritic cells induce different lineages of T cells, such as Th1, Th2, and Treg cells, which suppress the development of allergy and asthma, thus providing anti-inflammatory responses and protective immunity. DNA methylation regulates the differentiation of human monocyte-derived dendritic cells, affecting the cells to become either activated or tolerogenic [40]. In CD4+ cells, Treg cells maintain the demethylation status of TSDR and retain the ability to reactivate *FOXP3* expression and its suppressive function upon activation. TSDR changes when patients undergo treatment, but its cause or effect remains unknown for food allergy [40]. In a clinical trial, 20 out of 23 participants developed tolerance to 4 g of peanut protein after 24 months of oral immunotherapy. This was accompanied by an increase in antigen-induced Treg and a greater suppressive function due to elevated levels of *FOXP3* methylation. After withdrawal from the treatment for 6 months, 4 participants regained peanut sensitivity along with increased methylation of *FOXP3* CpG sites in antigen-induced Tregs [32]. The data were consistent and showed that immunotherapy altered the *FOXP3* methylation level to achieve the therapeutic effect.

A mice study revealed that epicutaneous immunotherapy could reduce the risk of further sensitization to peanut [43]. The mice were first sensitized with milk, followed by epicutaneous treatment for 8 weeks, and lastly they were sensitized to peanut. The study found an increase in the methylation level of the *GATA-3* promoter, which is located in the promoter region of *IL-5* and *IL-13*. Further to this, the study also showed that the methylation status was sustained over 2 months. Therefore, this study suggested that epicutaneous immunology modified the *GATA-3*

methylation level to inhibit further allergic sensitization to other allergens. Another study noted that epicutaneous immunotherapy appeared to be more effective compared with oral immunotherapy and a sustainable treatment for food allergy sensitization [44]. After 4 weeks of epicutaneous immunotherapy and oral immunotherapy, mice developed tolerance to peanut, accompanied by the occurrence of *FOXP3* demethylation in CD4⁺ T cells. However, following the end of treatment, only the *FOXP3* demethylation level in mice treated with epicutaneous immunotherapy remained persistent [44]. The persistent low *FOXP3* demethylation level was suggested to be essential for outgrowing the peanut sensitivities.

A study showed that the efficacy of antigen-specific immunotherapy could be increased through DNA methylation [43]. In a food allergy study with mice, the mice were divided into 2 groups: half of the mice were treated with allergen-specific immunotherapy while the others were treated with both allergen-specific immunotherapy and recombinant IL-8 [45]. The findings indicated that IL-8 significantly enhanced the therapeutic effect of allergen-specific immunotherapy and suppressed allergy inflammation via the promoter demethylation. Yang et al. [46] developed a food allergy mouse model in order to examine the relationships of IL-13, tolerogenic dendritic cells, and the development of food allergy. The mice were divided into 2 groups: a group of mice were treated with modified ag-specific immunotherapy (MSIT) alone, while the other group of mice were treated with MSIT and anti-IL-13 antibody. The mice were orally challenged and sacrificed the next day. The study showed that there was a significant difference in inflammation due to the allergy in mice treated with MSIT and anti-IL-13 antibody compared to mice treated with MSIT alone. IL-13 increased the methylation of the TSP1 (thrombospondin-1) gene DNA in B cells, which increased the development of tolerogenic dendritic cells in mice with food allergy and inhibited allergic inflammation in the intestine. These studies suggest that interleukins can improve the efficacy of allergen-specific immunotherapy by suppressing the allergic inflammation.

Diet is also a promising method for accelerating tolerance acquisition in children with cow's milk allergy [42]. In a clinical trial, 260 children were divided into the following 5 groups based upon the formula used for treatment: group 1 (extensively hydrolyzed casein formula), group 2 (extensively hydrolyzed casein formula and *L. rhamnosus*), group 3 (hydrolyzed rice formula), group 4 (soy formula), and group 5 (amino acid-based formula) [42]. After 12 months of exclusion diets with the different

formulas, the groups receiving extensively hydrolyzed casein formula (43.6%) or extensively hydrolyzed casein formula and *L. rhamnosus* (78.9%) had a higher rate of achieving oral tolerance compared to groups receiving hydrolyzed rice formula (32.6%), soy formula (23.6%), and amino acid-based formula (18.2%). The underlying mechanism was related to an increase in *FOXP3* TSDR demethylation as well [42]. Thus, CpG methylation within *FOXP3* appears to be a key therapeutic target for different types of food allergies. Considering that the observed changes in methylation occurred in multiple genes, other therapeutic targets should also be considered and evaluated in order to achieve long-term and synergistic effects.

Clinical Utility

The analysis of DNA methylation-based biomarkers is a rapidly developing field. Since the DNA methylation profile can be inherited from one generation to next, it may have potential to predict an offspring's susceptibility to disease by examining the parent's genome [47, 48].

DNA methylation may be suitable as a diagnostic signature for food allergy in 3 ways. Firstly, DNA methylation levels can be used to discriminate phenotypes of food allergy as well as other allergic diseases [49, 50]. In an Australian infant cohort study, it was discovered that the DNA methylation signature of 96 CpG sites could distinguish between food-allergic, food-sensitized, and non-food-allergic individuals, and predict clinical outcomes for food allergy [49]. The authors performed genome-wide methylation profiling on blood mononuclear cells from infants aged 11–15 months who underwent concurrent skin-prick testing and oral food challenges. Reproducibility was assessed with an additional 12 case and control samples, respectively, using methylation data from a publicly available dataset. This study found that DNA methylation in *MAP* kinase signaling genes was more accurate in predicting food challenge outcome compared to allergen-specific IgE and skin prick and had a 79.2% accuracy rate.

Secondly, certain DNA methylation signatures can be used to differentiate people with food tolerance from those with food intolerance. The gold standard diagnostic test in food allergy is double blind oral food challenge [51]. However, oral food challenge requires the food-sensitized children to produce an IgE-mediated response to antigens before food sensitization is confirmed. This causes threatening anaphylaxis to the children and makes

the parents stressful. As discussed earlier, *FOXP3* methylation can be used to discriminate tolerance and intolerance to peanut [32]. In this way, *FOXP3* methylation is a better way to diagnose food sensitization compared to oral food challenge, as this does not cause any discomfort to the patients with food allergy. Apart from *FOXP3* methylation, the DNA methylation level of selected Th1 and Th2 cytokines genes can be used as potential biomarkers in predicting tolerance acquisition. In a clinical trial, it was observed that healthy subjects had different DNA methylation profiles in selected Th1 and Th2 cytokines, such as *IL-4*, *IL-5*, *IL-10*, and *IFN-γ*, if compared to active cow's milk allergy patients [22]. In this way, DNA methylation analysis was clearly able to separate active cow's milk allergy patients from healthy controls.

Finally, with the aid of genome-wide technologies such as DNA methylation microarrays, high-throughput screening of patient samples becomes feasible and can be developed into prognostic markers, as some DNA methylation sites correlate with disease development and clinical outcomes [50].

There is a growing consensus that the development of food allergy is likely to be caused by epigenetic disruptions and the information of food allergy can be transmitted from one generation to the next (transgenerational effects). Maternal heredity is suggested to be a stronger determinant of allergy risk than paternal heredity, and allergic women are more likely to have infants with food allergy [52]. This was demonstrated through a birth cohort study, in which food allergy (positive food SPT and history of IgE-mediated symptoms) was shown to occur in 13% of 1-year-old infants of atopic (SPT+) women compared to only 4% of infants of nonatopic (SPT-) women [6]. This study suggests that allergic women have modified immune interactions with their fetus during pregnancy.

The development of food allergy is likely to be caused by epigenetic disruptions. Considering that DNA methylation is one of these epigenetic disruptions, it might be linked with the development of food allergy. Further studies are needed to confirm the role of DNA methylation as a causal mediator of food allergy.

Environmental Factors Affect DNA Methylation in Food Allergy

The rise in maternal allergy may also be affected by environmental changes. Around 13% of allergic women recruited after 2005 had a child with IgE-mediated food

allergy at 1 year of age, compared with 9% infants from a cohort of allergic women recruited in 1999 [6].

The maternal diet during pregnancy might alter DNA methylation and gene expression, and affect allergy outcomes in the offspring. A mouse study showed that maternal peanut allergy plus nontoxic cholera toxin subunit B consumption induced peanut tolerance in mice [53]. Mothers with peanut allergies were divided into 2 groups: 1 group was administered with low doses of peanut and nontoxic cholera toxin subunit B during pregnancy and lactation, while the other group was administered with water to serve as controls. The offspring of these mothers was challenged with peanut after 8 weeks of peanut sensitization. Following the challenge, there were no anaphylactic symptoms for the offspring of the treated mothers while the offspring of mothers who were administered with water showed anaphylactic symptoms. The newborn mice of the treated mothers showed a significant decrease in plasma histamine, peanut-IgE, IL-4, and IL-17, and a significant increase in IL-10 production compared to the newborn mice of the mothers that were administered with water. In addition, they showed a significant increase in CD4+CD25+Foxp3 T regulatory cell methylation. This suggested the increased peanut tolerance was due to nontoxic cholera toxin subunit B consumption which modified the expression of plasma histamine, peanut-IgE, IL-4, IL-17, and IL-10 through DNA methylation.

Vitamin D deficiency in pregnancy may be another risk factor for developing food sensitization through the suppression of Treg cells [54]. Two groups of female mice were fed different diets, 1 group a vitamin D-deprived diet and the other group a balanced diet. The female mice of each group were mated and continued the same diet during gestation and lactation. The offspring from these 2 groups were injected with ovalbumin and aluminum hydroxide to establish an animal model of food allergy at the age of 6 weeks. It was shown that the offspring of mothers that fed on the vitamin D-deprived diet had a higher risk of developing food allergies and a lower expression of Treg cells compared to the offspring of mothers fed on the balanced diet. Since a stable expression of Treg depends on the *FOXP3* DNA methylation, low vitamin D might reduce the Treg population through a change in methylation of *FOXP3* and result in increased susceptibility to the development of food allergy. In contrast, a LINA (Lifestyle and Environmental Factors and Their Influence on Newborns Allergy Risk) cohort study showed that high vitamin D levels in pregnancy and at birth increased the risk for developing food sensitization

[55]. In the study, 629 mother-child pairs were examined. Blood samples were collected from the mother at the 34th week of pregnancy, at delivery, and annually after birth. Tregs in cord blood were detected by methylation-specific PCR using a Treg-specific demethylated region in the *FOXP3* gene. A negative correlation between vitamin D and Tregs in the cord blood was identified, and low Treg numbers were associated with a higher likelihood of developing food allergy. The high amount of vitamin D was believed to suppress the Treg expression through the methylation of *FOXP3*, which led to a higher risk of developing food allergies. Evidently, the association of vitamin D with food allergy is controversial, and needs further investigation.

The composition of the microbiota may also contribute to the development of food sensitization [56–59]. Among all of the microbiota, *Bifidobacterium*, *Bacteroides*, and *Staphylococcus* have a protective impact towards milk allergy in mice [57, 58]. A mice study revealed that Ruminococcaceae (belonging to the system of Clostridiales) might be associated with IL-22. The study found higher levels of IL-22 and higher abundances of Ruminococcaceae in tolerant mice compared to allergic mice. Ruminococcaceae was suggested to be able to increase induced IL-22, which had a protective impact on food allergy in mice [60]. Microbiota composition can affect the DNA methylation in host cells [61]. The gut microbiota produces large amounts of epigenetically active metabolites, such as folate and butyrate, which may cause changes in the DNA methylation patterns of host cells, resulting in allergy development. Folate is essential for DNA methylation and acts as a methyl donor for the regeneration of the intracellular methyl substrate S-adenosyl methionine. A recent Finnish study indicated that folate intake and folic acid supplement use during pregnancy were associated with an increased risk of a cow's milk allergy in the offspring [62]. Folate acid altered the DNA methylation pattern which in turn increased the susceptibility to cow's milk allergy [52]. Butyrate is a short-chain fatty acid and it can be produced by *Faecalibacterium prausnitzii*, which belongs to the cluster of Firmicutes bacteria [63]. In a clinical trial, after 6 months of dietary intervention with extensively hydrolyzed casein formula supplemented with *L. rhamnosus* GG, it was observed that children who have tolerated cow's milk allergy presented a significant increase in fecal butyrate levels compared to children with cow's milk allergy [64]. Interestingly, another study also found that children with cow's milk allergy became tolerant after receiving a dietary treatment with extensively hydrolyzed casein formula containing the probi-

otic *L. rhamnosus* GG. It was found that the tolerance development was caused by increased *FOXP3* TSDR demethylation [33]. Therefore, these 2 studies suggest a potential link between butyrate, *FOXP3* TSDR demethylation, and the development of allergic disease. In other words, butyrate that is produced by microbiota can help to achieve tolerance by reducing *FOXP3* TSDR methylation.

In Australia, there is a remarkable increase in food allergy for new immigrants from Asian countries, and in particular the second generation of immigrants are 3 times more likely to develop nut allergy compared to the local children [65]. The genetic predisposition in these children implies that epigenetic changes occur in their parents when they are exposed to their new environment, and that these epigenetic modifications are inherited by the next generation. In our recent study, we showed that Chinese immigrants living in Australia for a longer period have increased global genome methylation levels [66]. Several environmental and lifestyle factors are suggested to cause the increased risk of allergies in Westernized countries, including exposures to new allergens or environmental microbiomes, Western diet and lifestyles, ultraviolet radiation exposure, and housing conditions [65]. Thus, multiple environmental factors may contribute to the development of food allergy through DNA methylation mechanisms.

Conclusion

DNA methylation modulates the development of the innate and adaptive immune systems, and plays an essential role in the development of allergic diseases. This research field is still in its infancy, particularly in relation to food allergy. Although further studies are clearly needed to determine how specific disease processes are mediated by DNA methylation, the currently available literature indicates the importance of DNA methylation in the development and manifestations of food allergy. It provides a new paradigm of allergy intervention, treatment and diagnosis. The DNA methylation-related technologies are developing rapidly, measuring increasing numbers of CpG sites as well as single-cell methylome sequencing. We can anticipate that these emerging technologies will greatly help us to understand the biologic and immunologic role of DNA methylation in food allergy, and will identify novel targets for clinical use.

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