



Genetics and epigenetics of allergy

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Purpose of review

Allergic diseases are prototypic examples for gene $\times$ environment-wide interactions. This review considers the current evidence for genetic and epigenetic mechanisms in allergic diseases and highlights barriers and facilitators for the implementation of these novel tools both for research and clinical practice.

Recent findings

The value of whole-genome sequencing studies and the use of polygenic risk score analysis in homogeneous well characterized populations are currently being tested. Epigenetic mechanisms are known to play a crucial role in the pathogenesis of allergic disorders, especially through mediating the effects of the environmental factors, well recognized risk modifiers. There is emerging evidence for the immune-modulatory role of probiotics through epigenetic changes. Direct or indirect targeting of epigenetic mechanisms affect expression of the genes favouring the development of allergic diseases and can improve tissue biology. The ability to specifically edit the epigenome, especially using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 technology, holds the promise of enhancing understanding of how epigenetic modifications function and enabling manipulation of cell phenotype for research or therapeutic purposes.

Summary

Additional research in the role of genetic and epigenetic mechanisms in relation to allergic diseases' endotypes is needed. An international project characterizing the human epigenome in relation to allergic diseases is warranted.

Keywords

allergic diseases, asthma, biomarkers, epigenetics, genetics, prevention, risk stratification

INTRODUCTION

Successful management of patients with allergic diseases continues to be a major unmet need. One of the barriers is their heterogeneity conferred by an intricate network of risk factors, visible properties (phenotypes) and outcomes depending on different mechanisms (endotypes), driven by innate and adaptive immune responses and continuously modulated by the micro-environment and macro-environment [1]. The strong familial clustering has encouraged research into the genetic predisposition and recent decades have witnessed an explosion of gene variants associated with disease risk. Allergic diseases are polygenic, multifactorial disorders, where the combined action of several genes interacting with one another and with environmental factors drives disease patho-mechanisms [2,3^{*}]. It has become clear that understanding epigenetics and epigenomics is essential for a better prediction of allergy risk and its natural history. Despite the potentially huge role that epigenetics may play in

allergic diseases, research in this area lags behind compared with that devoted to genetics research [3^{*}].

THE CURRENT LANDSCAPE OF THE GENETICS AND EPIGENETICS RESEARCH IN ALLERGY

The evaluation of the genetic risk in complex diseases such as asthma, allergic rhinitis (AR), chronic rhinosinusitis (CRS), atopic dermatitis (AD), food allergy (FA) or eosinophilic esophagitis (EE) is not straightforward. The genetic basis has been extensively investigated in the past decades using linkage

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KEY POINTS

- Despite its success, discoveries from genome-wide association studies to date have contributed relatively little to understanding specific causal genetic mechanisms underlying allergic diseases.
- Epigenetic marks are emerging as mediators of genetics and the environment impact driving complex disease phenotypes, such as allergic diseases and asthma.
- Identification of key epigenetic marks and their impact on transcription of specific genes will lead to a better understanding of cause, heterogeneity and severity of allergic diseases, and will also facilitate targeted interventions, both for prevention and for treatment.
- Epigenetic and transcriptomic profiling may yield the most accurate risk estimates for a given patient by incorporating environmental, including treatment, effects throughout the lifespan.
- To further support the gene-environment-wide interaction studies an international project characterizing the human epigenome in relation to allergic diseases and asthma is warranted.

analysis and candidate-gene association studies. Following the development of genetic tools genome-wide association studies (GWAS) have become ‘the norm’ (Table 1), however, the trade-off between larger sample sizes to increase power and focus on specific subtypes (severe or childhood-onset asthma or highly specific food allergies) to decrease clinical heterogeneity is not yet settled [3[■],4,5,6[■],7–10].

The epigenome has an instrumental role in determining cell identity and function, while its dysregulation leads to aberrant gene expression and disease. Epigenetic dysregulation can induce both short-term and long-term changes, starting *in utero*, varying throughout the life span, and in some instances inherited. The analysis of epigenetic modifications has attracted substantial interest as related to environmental risk or protective factors heavily impacting the natural history of allergic diseases. Most of the research on DNA methylation (DNAm) evaluated whole blood or peripheral blood mononuclear cells (PBMCs). Alterations in DNAm seen in whole blood can be reproduced with purified eosinophils with a larger magnitude of effect, with an ‘eosinophilic signature’ characteristic for allergic diseases [11,12[■],13]. For airway epithelial cells (AEC) there is only a small overlap with whole blood [11,14]. An AEC cell-specific signature was recently described [14]. Several other cell-specific monocytes, B lymphocytes, airway smooth muscle cells (ASM) DNAm

studies were performed [15,16]. Cell-specific DNAm studies facilitate the evaluation of proxies, such as whole blood, buccal or nasal samples [17[■]].

The microbiome assembles during the first year of life and critically shapes the microbiome and the healthy immune responses and metabolic processes later in life. The microbiome and related metabolites are capable of epigenetic modulation. Interactions between the host epigenome and the microbiome (the hologenome) are increasingly recognized. For inflammatory bowel diseases an epigenetic profile primed by commensal microbes was recently described and correlated with disease severity [18]. Both obesity or asthma induce changes in the composition of the microbiota, with an additive effect in obese asthma [19[■]].

GENETIC AND EPIGENETIC RISK FACTORS

The clinical utility of polygenic risk score analysis (PRS) was not validated across ethnic/racial groups [20]. Distinct patterns of genetic associations with disease phenotypes were recently shown, suggesting heterogeneous mechanisms underlying individual disease trajectories [21]. Whole-genome sequencing studies in well characterized populations such as childhood-onset asthma or asthma with co-morbidities are currently set-up. The evaluation of the PRS in association with asthma characteristics showed for patients with childhood-onset asthma and high genetic risk a higher likelihood to develop life-course-persistent asthma [22]. In addition, PRS may be of value when evaluating the shared genetic predisposition for allergic diseases. Robust and significantly higher PRSs of asthma were observed in children suffering from cow’s milk allergic [23].

Epigenetic marks, especially DNAm and micro-RNAs (miRNAs)], have the potential for assessing the risk of development of atopic sensitization or allergic disease. Over the past years epigenome-wide association studies (EWAS) have added to the growing body of evidence supporting significant associations of epigenetic regulation of gene expression and allergy risk.

The cross-sectional association between the epigenome-wide DNAm in peripheral blood and allergic diseases was investigated in two birth cohorts. Different epigenetic risk profiles for mid-childhood atopic sensitization, inhalant and food allergen sensitization were described [24]. Placental histone acetylation at the promoter regions of allergy-related immune regulatory genes was described as a risk factor for sensitization to food or inhaled allergens [25]. Histone-acetyltransferase (HAT) activities are required in gene transcription of a large number of immune molecules. HAT Tat-interactive protein 60

Table 1. Recent genetic studies in allergic diseases

GWAS	Comments	Key findings	Reference
Asthma	Meta-analysis of GWAS	Overlap with diseases such as neoplasia, cardiovascular, neuropsychiatric	Demendis <i>et al.</i> Nat Genet 2018;50:42–53
	The largest GWAS of asthma [23 948 patients with European, African, Japanese, Latino ancestry Combined individual GWASs within and across 4 groups included in the Trans-National Asthma Genetic Consortium	Enriched in regions known as enhancers in immune cells Overlap with risk regions for decreased lung function, allergic or immune-related diseases 2 new loci at regions associated with asthma and AR	
GWAS according to age of asthma onset	3 GWASs: asthma onset before 12 years old (childhood onset), asthma onset between 26 and 65 years (adult onset) and one considering asthma age of onset as a quantitative trait	23 Loci specific to childhood-onset asthma, 1 specific to adult-onset asthma, and 37 shared	Pividori <i>et al.</i> Lancet Respir Med 2019;7:509–22
		Genes at childhood-onset loci highly expressed in skin (epithelial barriers endotype) Genes at adult-onset loci were more highly expressed in the lung and spleen (lung centered, immune mediated endotype)	
GWAS of moderate-to-severe asthma	GWAS with a special focus on signals meeting genome-wide significance and asthma severity	Variants near MUC5AC contribute to the more severe presentation in adult-onset asthma	Shrine <i>et al.</i> Lancet Respir Med 2019;7:20–34
GWAS for specific features of asthma (e.g. airway hyperreactivity)	Gene-environment GWAS post diesel exhaust particles	Adaptor molecule expressed in immune-related and mucosal tissues, including the lung	Maazi <i>et al.</i> PLoS Genet. 2019; 15(12):e1008528
GWAS for asthma and comorbidities (e.g. obesity)	cross-trait GWAS on 457 822 patients of European ancestry from the UK Biobank	Positive genetic correlation between BMI and late-onset asthma	Zhu <i>et al.</i> J Allergy Clin Immunol. 2019 Oct 24. pii: S0091–6749(19)31366–1. doi:10.1016/j.jaci.2019.09.035. [Epub ahead of print]
		Shared immune-related and cell differentiation-related pathways between obesity and asthma	

Table 1 (Continued)

GWAS		Comments	Key findings	Reference
AR	Meta-analysis of GWAS	The largest GWAS of AR including 18 independent trials of European ancestry	41 independent loci, 20 new risk alleles	Waage <i>et al.</i> Nat Genet 2018;50:1072–80
AD	Meta-analysis of GWAS	26 Multiethnic studies (European, African, Japanese and Latino ancestry)	31 Risk loci, 10 new loci Only 3 of them IL13, IL-6 receptor, and filaggrin resolved to protein-coding variants	Paternoster <i>et al.</i> Nat Genet. 2015;47(12):1449–1456
FA	GWAS for specific foods	Hydrolysed wheat protein Shrimp Peach Peanut	MHC-related risk alleles for food allergy are independent of the MHC-related risk alleles for asthma	Noguchi <i>et al.</i> J Allergy Clin Immunol 2019;144:1354–63
Allergic disease risk	GWAS of a broad allergic disease phenotype that considers the presence of any one of asthma, AR and AD		Risk locus near the risk region for AD, asthma, and inflammatory bowel disease	Khor <i>et al.</i> Sci Rep 2018; 8:1069 Asay <i>et al.</i> Nat Genet 2018;50:1072–80
			Tissue-specific heritability and biological process enrichment analyses suggest that shared risk variants influence lymphocyte-mediated immunity For 36 genes CpG methylation influences transcription independently of genetic effects	Ferreira <i>et al.</i> Nat Genet. 2017;49(12) 1752–1757
	Previous GWAS adjusted results	The EUGENE gene-based approach was used, combining evidence for association with disease risk across regulatory variants identified in different tissues	11 New risk loci	Ferreira <i>et al.</i> J Allergy Clin Immunol. 2019;143(2):691–699

AD, atopic dermatitis; AR, allergic rhinitis; GWAS, genome-wide association studies.

(Tip60) is required for the expression of IgE and IgG1 in B cells by inducing chromatin remodelling, while blocking Tip60 significantly attenuated the allergic inflammation [26].

Most human studies of the epigenomics of asthma have focused on cytosine and guanine separated by only one phosphate group (CpG) DNAm in whole blood, PBMCs, AECs or in the nasal epithelium [11,12[■],14,27,28,29[■],30]. DNAm frequently reported are related to epithelial barrier or to immune regulatory processes. Characteristic alterations in DNAm were associated with inflammatory phenotypes of asthma and may contribute to the disease mechanisms [31]. Showing robustness of results across populations from different racial/ethnic backgrounds exposed to diverse environments the nasal respiratory epithelium methylome is a good proxy for asthma risk [29[■]]. A recent paediatric EWAS evaluating DNAm patterns in nasal swabs found multiple differentially methylated regions (DMRs) for allergic asthma, exhaled nitric oxide (FeNO) total and specific IgE, and bronchodilator response. The identified DMRs were related to genes implicated in allergic asthma, Th2 activation and eosinophilia. Asthma, IgE and FeNO were associated with nasal epigenetic age acceleration [17[■]]. Emerging data involve miRNA in asthma as drivers of lung macrophage polarization, of epigenetic dysregulation during asthma exacerbations leading to lung function impairment, airway inflammation and T-cell cytokine expression or facilitating viral-induced exacerbations [32–34]. Several exposures have been linked to the observed epigenetic changes in asthma such as environmental tobacco smoke, other pollutants, diet, viral infections, the lung/gut/nasal microbiome and the type 2 cytokines IL-4 and IL-13 impacting epithelial barrier integrity [35–38,39,40]. These exposures mainly act into the ‘critical window of risk’ for the immune system and the resident cells, during the foetal development and in the first year after birth. The respiratory syncytial virus (RSV), an omnipresent virus that infects nearly all children by age 2 years and the most common cause of hospitalization in children under 2 years of age, causing severe lower respiratory tract infection, bronchiolitis and pneumonia is associated with an increased risk of developing childhood asthma and recurrent wheezing [42]. In severe RSV infections inefficient innate immune and excessive type 2 and T17 responses are described [41,43–46]. Children hospitalized with severe RSV-induced bronchiolitis as infants had decreased methylation of the perforin-1 enhancer, indicating a defect in natural killer (NK) cells antiviral intervention [47]. Allergic mice infected with RSV develop steroid-insensitive inflammation driven by the upregulation of miRNA inhibiting steroid responsiveness [48]. RSV infection

of dendritic cells leads to alterations in histone methylation with decreased proinflammatory cytokine production and subsequent increase of type 2 cytokines [49]. Transcriptome analysis of constitutive gene expression in AECs revealed restrictive and permissive patterns of cell response to RSV infection [50].

For atopic dermatitis striking DMRs were observed between lesional and healthy control skin, partly correlated with altered transcription of genes relevant for epidermal differentiation and innate immune response [51]. A CpG in IL-4 associated with total IgE was recently convincingly replicated, supporting a role for epigenetic IL-4/IL-13-induced type 2-immune mediating mechanisms in AD [52]. Hypomethylation of thymic stromal lymphopoietin and of the high-affinity receptor for IgE were reported together with significantly over-expressed miR-155, targeting the immune suppressor CTLA-4 [53–55].

Single-cell RNA sequencing in CRS showed distinct transcriptomes for respiratory epithelial, immune and stromal cell types and type 2 inflammatory subsets. Cell-intrinsic, epigenetic and extrinsic factors lock polyp basal cells into an uncommitted state. Ex-vivo cultured basal cells retain intrinsic memory of IL-4/IL-13 exposure, with a potential of blockade of the IL-4 receptor α -subunit (e.g. dupilumab) to modify basal and secretory cell states [56]. Decreased DNAm of the IL8 proximal promoter might play a role in the pathogenesis of CRS with nasal polyps [57].

The interplay of gene (*CAPN14* and *LOC283710/KLF13*) and early-life environment factors contribute to eosinophilic esophagitis susceptibility through epigenetic mechanisms [58,59].

The methylation process may serve as an anchor upon which gene expression modulates disease severity. DNAm linked to low lung function were identified both in smokers and in nonsmokers [59,60]. DNAm modules in ASM involved in contraction (calcium signalling pathway), proliferation (cancer-related pathways), the endocytosis pathway and the endocannabinoid signalling were associated with asthma severity. DNAm also impacted the expression of miRNA further influencing ASM function [16]. Following allergen provocation of allergic rhinitis patients in the exposure chamber DNAm significantly correlated with the occurrence symptoms, while baseline DNAm pattern was predictive of symptom severity. Changes in mucine 4 DNAm correlated with the drop in peak nasal inspiratory flow [61]. A recent transcriptomic and epigenomic study of peanut allergic children following double-blind, placebo-controlled peanut challenges identified differential DNAm associated with reaction severity. The ‘peanut severity genes’ were related

to neutrophil-mediated immunity, chemotaxis and regulation of macroautophagy [62].

BIOMARKERS FOR EARLY INTERVENTION

Although still in the early phase, epigenetic markers, particularly DNAm and miRNAs, might have potential for stratification of patients for treatment and complement or replace in the future biochemical or clinical tests.

A large-scale EWAS within the Mechanisms of the Development of ALLergy project showed that reduced whole-blood DNAm at 14 CpG sites acquired after birth is strongly associated with childhood asthma [11]. These CpG sites and their associated transcriptional profiles indicate activation of eosinophils and cytotoxic T cells. Lung function-associated methylation signals in adults are possibly of clinical utility in identifying poor lung function and accelerated decline [59]. Epigenetic regulation of human β -defensin-1 (HBD-1) might be associated with a variety of defects in the innate immune system during atopic dermatitis pathogenesis. Significantly higher methylation frequencies at the CpG 3 and 4 sites were demonstrated in atopic dermatitis lesional skin compared with nonlesional and normal skin. Thus, DNAm signature of HBD-1 could become a novel diagnostic/prognostic marker and a promising therapeutic target for the compromised atopic dermatitis barrier attributed to HBD-1 deficiency [63].

DNAm can be a predictor of responses in controlled allergen challenges [61,64]. Epigenetic biomarkers correlating with successful outcome of allergen immunotherapy (AIT) have been reported. DNAm of promoter regions such as Forkhead box protein 3 (Foxp3) are associated with clinical reactivity and can be changed following AIT [64,65]. The epigenetic regulator enhancer of zeste homolog 2 was shown to be a critical factor in the activation of dendritic cells following AIT [66]. IL13 promoted thrombospondin-1 gene methylation in B cells can be reduced following oral immunotherapy [67,68].

POTENTIAL FUTURE TOOLS

GWAS evaluating racial/ethnic groups that carry the greatest burden of allergic disease remain significantly underrepresented [69]. A separation between disease susceptibility, natural evolution and severity and response to treatment together with novel tools such as reverse phenotyping using genetic markers to define endotypes and integration of multiomics measures can largely improve the outcome of genetic and epigenetic studies [70]. A computational machine learning approach using DNAm data was recently tested for food allergy [71]. Functional and causal gene variants may link genetic risk to disease phenotype and endotype. The study of rare coding variants might also prove useful, as already demonstrated for atopic dermatitis [72].

The epigenome provides an interesting target for therapeutic intervention. Compared with genome editing, epigenome editing and transcriptional modulation offer several advantages in biomedical research and development of precision medicine for allergic diseases (Table 2).

Three molecular platforms for epigenetic editing have been developed: zinc finger proteins, transcription activator-like effectors, and the system of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins [73]. These platforms serve as DNA-binding domains fused with epigenetic modifying domains. The addition and/or removal of epigenetic modifications reconfigures local chromatin structure, with the potential of long-lasting changes in gene transcription. CRISPR/Cas9-based RNA-guided DNA endonuclease technology using the nuclease-dead Cas9 (dCas9) that allows binding of Cas9 to target DNA without inducing cleavage has become the preferred platform for epigenome editing [74–76]. Using this technology stable and heritable alteration of epigenetic marks and gene expression can be achieved in vivo in postmitotic tissues without the need to modify the DNA sequence. Recent advances in adeno-associated virus delivery of CRISPR fusion proteins together with RNA targeting strategies

Table 2. The advantages of epigenome editing and transcriptional modulation

Targeted epigenetic-oriented therapy can be used without the need to genetically modify the mutated DNA sequence or inducing DNA damage the potential creation of unwanted mutations within the target locus and off-target effects is minimized
Allergic diseases and asthma are polygenic in origin and associated with epigenome dysregulation. Epigenetic therapy can provide a better therapeutic efficacy by simultaneous modulation of multiple genes
Sustained transcriptional activation of endogenous cell master transcription factor with a more natural cell lineage conversion than forced overexpression of reprogramming factors via genetic editing, in addition providing insights into the molecular mechanism of cellular reprogramming
Stable or reversible manipulation of epigenetic states, depending on the types of catalytic domain or inducible strategy used
Long-term expression of chromatin modifiers or transcriptional regulators in postmitotic tissues without the need to integrate the transgenes into the host genome

Table 3. Interventions on the microbiome for allergic diseases and asthma: the effects of probiotics

Study	Key findings	Reference
Randomized double-blind placebo-controlled trial, <i>Lactobacillus</i> (L.) <i>casei</i> and <i>Bifidobacterium</i> (B.) <i>lactis</i> added to hypoallergenic formula for 12 months	Did not modulate the rate of immune tolerance acquisition to cow's milk proteins in infants with CMA	Hol <i>et al.</i> <i>Allergy Clin Immunol.</i> 2008; 121:1448–54
EHCF versus EHCF containing LGG 12 months	The double-blind placebo-controlled food challenge was negative 53.6% controls and in 81.5% of the LGG group	Berni Canani <i>et al.</i> <i>J Allergy Clin Immunol.</i> 2012; 129:580–2
Children with IgE-mediated CMA randomly allocated to the EHCF or EHCF + LGG groups and followed for 3 years for the occurrence of AD, urticaria, asthma, or rhinoconjunctivitis. The secondary outcome was tolerance acquisition through double-blind food challenge results at 12, 24, and 36 months	The absolute risk difference for the occurrence of at least one allergic disease over 3 years was -0.23 (95% CI, -0.36 to -0.10 ; $P < 0.001$). The absolute risk difference for the acquisition of cow's milk tolerance was 0.20 (95% CI, 0.05 – 0.35 ; $P < 0.01$) at 12 months, 0.24 (95% CI, 0.08 – 0.41 ; $P < 0.01$) at 24 months, and 0.27 (95% CI, 0.11 – 0.43 ; $P < 0.001$) at 36 months	Berni Canani <i>et al.</i> <i>Allergy Clin Immunol</i> 2017; 139(6):1906–1913
Children with eczema and/or CMA who received EHCF and LGG	Decreased skin inflammation and gastrointestinal symptoms	Baldassare <i>et al.</i> <i>J Pediatr.</i> 2010; 156:397–401
Two-centre randomized placebo-controlled trial of LGG or <i>B. lactis</i> taken daily from 35-week gestation to 6 months' postpartum in mothers while breastfeeding	LGG significantly protected against eczema development at 2, 4, 6, and 11 years and atopic sensitization at 6 years. No effect of <i>B. lactis</i>	Wickens <i>et al.</i> <i>Pediatr Allergy Immunol.</i> 2018; 29(8):808–814
Double-blind, placebo-controlled intervention trial, children randomized to daily mixture of LGG and <i>B. animalis</i> subsp <i>lactis</i> or placebo-starting prior to attending day care. Intervention period 6 months	Significantly lower incidence of eczema in the probiotic group compared with the placebo group	Schmidt <i>et al.</i> <i>Pediatr Allergy Immunol.</i> 2019;30(3):335–340
Randomized double-blind placebo-controlled trial in 62 children with peanut allergy treated with fixed dose of probiotic together with peanut OIT (PPOIT) or placebo once daily for 18 months No OIT control group included to distinguish the probiotic itself had any effect on SU	SU determined by double blind placebo-controlled food challenge was achieved in 82.1% of children receiving PPOIT compared with 3.6% of those receiving placebo. PPOIT also induced high rates of resolution (90%) and was associated with reduced skin prick test reactivity, decreased peanut-specific IgE and increased peanut-specific IgG4 levels. No participants withdraw because of adverse reactions	Tang <i>et al.</i> Administration of a probiotic with peanut oral immunotherapy: a randomized trial. <i>J Allergy Clin Immunol.</i> 2015; 135:737–44
100 AR children, ages 5–12 years, sensitive to grass pollen receiving 5-grass SLIT tablets with either vitamin D 1000 IU daily supplementation, probiotic, or placebo The control group included children with allergy who did not qualify for immunotherapy Primary end points included a symptom-medication score, lung function, and FeNO The secondary end point was the immunologic efficacy	In the SLIT-probiotic group between-group analysis showed significantly higher CD4(+)CD25(+)Foxp3(+) induction compared with the SLIT group and higher reduction in the percentage of TLR-positive cell group compared with the SLIT-vitamin D group	Jerzynska <i>et al.</i> <i>Allergy Asthma Proc.</i> 2016;37(4):324–34
Meta-analysis investigating the effectiveness of probiotic supplementation in pregnant women	No significant differences between probiotic and placebo for the incidence of allergic disease, asthma or atopic sensitization	Kuang; <i>Br J Nutr.</i> 2019:1–31

Table 3 (Continued)

Study	Key findings	Reference
Meta-analysis of 17 randomized controlled trials, 5264 children	Only LGG supplementation had a reduction in the occurrence of asthma No effect on wheeze, AR, atopic status (positive skin prick test)	Du <i>et al.</i> Allergy Asthma Proc. 2019;40(4):250–260
Meta-analysis of 19 randomized trials, 5157 children	Subgroup analysis by asthma risk showed that probiotics significantly reduced wheeze incidence among infants with atopy disease No effect on the overall risk of asthma	Wei <i>et al.</i> J Asthma. 2019;1–12

AD, atopic dermatitis; AR, allergic rhinitis; CI, confidence interval; CMA, cow's milk allergic; EHCF, Extensively hydrolysed casein formula; FeNO, exhaled nitric oxide; LGG, *Lactobacillus rhamnosus* GG; PPOIT, peanut OIT; SU, sustained unresponsiveness; SLIT, sublingual immunotherapy; TLR, Toll-like receptor.

greatly facilitate the in-vivo application of this tool [77,78]. Various catalytic domains of epigenetic effectors can be fused to dCas9 for studying the effects of a particular epigenetic regulation and gene-environment interaction. Epigenetic editing was tested for reducing mucus production in AECs, using both zinc finger proteins and CRISPR/dCas platforms. All constructs effectively suppressed SAM pointed domain-containing Ets transcription factor (SPDEF) mRNA and protein expression, with inhibition of downstream mucus-related genes. The obtained silencing was mitotically stable inducing long-lasting mucus inhibition [79].

The increased knowledge of the crucial role played by nutrients and gut microbiota-derived metabolites is opening the way to a postbiotic approach in the stimulation of immune tolerance through epigenetic regulation. In a mouse model of food allergy histone acetylation of Th2 genes was lower after exposure to raw milk compared with processed milk [80]. Children with the highest levels of short-chain fatty acids (SCFAs) in faeces at the age of 1 year had significantly less atopic sensitization, asthma, food allergy or allergic rhinitis [81]. The SCFAs secreted by the gut healthy microbiome influence epigenetically, mainly through HDAC inhibition, several nonimmune (tight junction proteins, mucus production) and immune cells involved in the immune tolerance network [82]. Butyrate and propionate decrease DNAm of IL-10 and IFN- γ genes, induces FoxP3 demethylation and down-regulation of HDAC6/HDAC9 expression [83,84]. In-vitro SCFAs were able to attenuate human eosinophils' adhesion to the endothelium, migration and survival. These effects were accompanied by histone acetylation and mimicked by a pan-histone deacetylase inhibitor. In-vivo butyrate ameliorated allergen-induced airway eosinophilia, reduced type 2 cytokines in bronchial fluid, and improved airway hyperresponsiveness [85]. On the contrary, most data on intervention with probiotics and prebiotics

come from in-vitro results or animal models. There seems to be a clinical benefit for food allergy and atopic dermatitis while for asthma and allergic rhinitis the evidence is less convincing (Table 3).

Selection of responders to probiotics is of utmost importance in the advent of personalized medicine. Unsupervised hierarchical clustering of systemic and nasal immune gene expression at baseline and after supplementation separated responders from nonresponders to probiotics in allergic rhinitis, with key T-cell immune genes differentially expressed [86].

From all these potential future tools in our view the quest for functional and causal gene variants linking genetic risk to disease endotype using mathematical models and the fast development of the epigenetic editing tools will significantly enrich the knowledge in the field of genetics and epigenetics of allergic diseases in quite a short period of time.

CONCLUSION

The ultimate goal of understanding the genetic and epigenetic mechanisms in allergic disease is to better match individualized interventions (both prevention and treatment) to specific endotypes to improve outcomes and minimize side effects. On the contrary, in the GWAS and EWAS era, a positive family history still remains a reliable tool for estimating the risk of allergic disease. There are high hopes from the targeted evaluation of well defined disease phenotypes, reverse phenotyping and the use artificial intelligence to build unbiased data-driven models.

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Conflicts of interest

There are no conflicts of interest.

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