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Review article

Epigenome-wide association studies of allergic disease and the environment

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The epigenome is at the intersection of the environment, genotype, and cellular response. DNA methylation of cytosine nucleotides, the most studied epigenetic modification, has been systematically evaluated in human studies by using untargeted epigenome-wide association studies (EWASs) and shown to be both sensitive to environmental exposures and associated with allergic diseases. In this narrative review, we summarize findings from key EWASs previously conducted on this topic; interpret results from recent studies; and discuss the strengths, challenges, and opportunities regarding epigenetics research on the environment-allergy relationship. The majority of these EWASs have systematically investigated select environmental exposures during the prenatal and early childhood periods and allergy-associated epigenetic changes in leukocyte-isolated DNA and more recently in nasal cells. Overall, many studies have found consistent DNA methylation associations across cohorts for certain exposures, such as smoking (eg, aryl hydrocarbon receptor repressor gene [*AHRR*] gene), and allergic diseases (eg, *EPX* gene). We recommend the integration of both environmental exposures and allergy or asthma within long-term prospective designs to strengthen causality as well as biomarker development. Future studies should collect paired target tissues to examine compartment-specific epigenetic responses, incorporate genetic influences in DNA methylation (methylation quantitative trait locus), replicate findings across diverse populations, and carefully interpret epigenetic signatures from bulk, target tissue or isolated cells. (J Allergy Clin Immunol 2023;152:582-90.)

Key words: *EWAS, asthma, allergy, atopy, DNA methylation, epigenetics, environment*

Abbreviations used
AHRR: Aryl hydrocarbon receptor repressor
CpG: Cytosine-phosphate-guanine
DMR: Differentially methylated region
EAA: Epigenetic age acceleration
EWAS: Epigenome-wide association study
FDR: False discovery rate
$PM_{2.5}$: Particulate matter less than 2.5 μm in diameter
SNP: Single-nucleotide polymorphism

Allergic diseases are a growing health concern worldwide, and recent studies have implicated epigenetic mechanisms with allergy. Epigenetic modifications are changes in gene expression not related to variations in the genetic code that can act as an interface between environmental exposures, lifestyle, and allergic diseases.[1-3] Asthma, allergic rhinitis, atopic dermatitis, and food allergy affect up to 20% of the world's population, and these prevalent diseases can significantly affect quality of life and cause substantial direct and indirect costs to society.[4] Lifestyle factors, such as dietary habits, delayed introduction of food allergens, and hygienic practices, as well as environmental factors, such as exposure to cigarette smoke, air pollution, microbes, and allergens (some of which can be aggravated by climate change), may all contribute to disease onset and severity.[5] Epigenetics research on the mechanisms and biomarkers of allergic disease may help elucidate how environmental exposures in the prenatal period and early life promote allergy throughout the life course and across generations.

Many scientists have proposed that the epigenome is key to understanding allergic disease, given both the role of the environment in allergic disease and robust evidence on the influence of some environmental factors on the human epigenome.[6-8] Over the past 10 years, numerous epigenome-wide association studies (EWASs) have been performed to investigate the link between environmental factors and allergic diseases and DNA methylation variation of different cells and tissues.[5,9] In this narrative review, we report and discuss recent findings within the context of the existing literature, with the goal being to answer the question: "Do these data support the concept of environmental exposures contributing to alterations in epigenetic markers related to allergy and asthma?" We have focused this report on EWASs of DNA methylation, as this method has comprehensively assessed allergic diseases and many environmental factors relevant to allergy in a systematic manner across human studies.

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We first provide background on EWAS approaches, describe our search methods, and discuss findings from major and recent EWASs on this topic. Then, we provide an interpretation of epigenetic findings, challenges, and recommendations, mention significant findings from epigenetic aging studies, and discuss potential future clinical applications.

BACKGROUND ON EWASs

The aim of EWASs is to examine epigenetic variation, usually mean changes, across the genome to detect differences in DNA methylation at a given cytosine-phosphate-guanine (CpG) dinucleotide in relation to an exposure or phenotype of interest.[10] First, DNA is extracted from a population of cells of different tissues and bisulfite-converted, which results in a change from unmethylated cytosines to uracil, whereas methylated cytosines remain unaffected. Subsequently, DNA is hybridized to oligonucleotides on microarrays, such as the 450K or 850K EPIC arrays, to investigate potentially methylated CpG sites across the genome. Array data are subsequently processed by using bioinformatic pipelines, and the percentage of methylated versus unmethylated DNA at a certain genomic position is subsequently tested against a phenotype or exposure of interest with various statistical models.[10] In addition to the investigation of differentially methylated and variable positions in the genome, several statistical techniques have been developed to evaluate differentially methylated regions (DMRs),[11] combining methylation data at adjacent CpG sites or modules of coregulated or correlated CpG sites.[12]

Most studies have been performed on DNA derived from cells and tissues of mixed origin, which has implications for downstream interpretation. A difference in DNA methylation at a certain CpG site may be due to changes of DNA methylation at that particular position within a cell type, differences in cell type composition (in which case a particular cell type is associated with CpG methylation at this locus), or a combination of both. To account for the effect of cell type composition on DNA methylation studies, it is now common practice to adjust for cell type estimates from the DNA methylation data when reference panels are available or to adjust for surrogate measures, computationally, when DNA methylation panels of target cells are not available.[13]

SEARCH METHODS

In addition to discussing key articles published on the topic of asthma and allergy EWASs that have been covered in previous reviews,[2,14-17] we evaluated more recent studies in this narrative review. The most recent systematic review on this topic included studies published from January 1, 2005, to February 1, 2019.[14] For this narrative review, we performed an updated online search for original research articles on asthma and allergy EWASs published between February 1, 2019, and August 30, 2022. The search was performed in 3 databases, PubMed, EMBASE, and Web of Science, by using combinations of search terms and Medical Subject Heading (MeSH) terms, including *epigenetics, asthma, allergy, DNA methylation*, and *EWAS* (Table I). In our interpretation, we focused on CpG sites that were replicated in independent studies for allergic disease and highlight important findings from environmental epigenetics that are relevant to allergic disease.

TABLE I. Description of the strategy used to search for recent articles

Search parameter	Details
Dates of search	February 1, 2019, to August 30, 2022
Databases	PubMed, EMBASE, Web of Science
Search terms	*Epigenetics, asthma, allerg*, allergic disease, atopic disease, rhinitis, eczema, food allergy, atop*, eosinophil*, IgE, DNA methylation, EWAS, epigenome-wide, air pollut*, environment, environmental exposure, environmental factor*

EWASs OF DNA METHYLATION RELATED TO ALLERGIC DISEASES

Several EWASs have been conducted to examine the associations between DNA methylation patterns and asthma and allergy to characterize the pathologic roles of epigenetic changes in these conditions. A review article on this topic that covered articles published until February 2019 included several EWASs on allergic diseases[18-22] and described heterogeneity across studies pertaining to the epigenetic laboratory techniques, participant age, use of replication cohorts, and type of specimen analyzed.[14] We found a similar degree of heterogeneity in the more recently published EWASs discussed below; however, due to advancements in the field, these studies highlight new and unique findings by using multiple replication cohorts, larger sample sizes, and more sophisticated analytical methods. The differentially methylated CpGs and corresponding gene annotations for each recently published EWAS are included in Table E1 (in the Online Repository at www.jacionline.org). These studies demonstrate replicated and novel epigenetic signatures of asthma in diverse populations, including individuals of Latino, Asian, and African American ancestries,[23-25] highlighting the importance of increasing diversity in EWASs. This is key to ensuring that novel omics-based biomarkers and treatments are equitably justified in diverse patients. Although epigenetic changes are cell type–specific, studies have reported overlap between results from samples of whole blood, nasal epithelium, and bronchial epithelium in the original study and replication cohorts, suggesting some common cross-tissue epigenetic alterations and pathologic changes.[7,26,27]

The majority of EWASs have focused on analyzing DNA methylation of whole blood cells in children with asthma, as determined by reported doctor's diagnosis, symptoms, and medication use. In analyses adjusted for proportions of cell types, age, and sex, children with asthma who were of Latino ancestry and aged 6 to 20 years were found to have DNA methylation profiles different from those of their counterparts without asthma.[23] The enrichment analysis was notable for TGF-β signaling, interferon signaling, and T_H1 and T_H2 cell activation pathways. A total of 25 genomic regions were differentially methylated; the top 2 CpGs were near the calcium/calmodulin-dependent protein kinase 1D (*CAMK1D*) and T-cell immunoreceptor with Ig and ITIM domains (*TIGIT*) genes, which have been implicated in inflammatory pathways and airway constriction.[24,28] A combined EWAS and genome-wide association study found that differential methylation of *CAMK1D* directly contributed to tissue eosinophilia and also reported a causal effect of changes in Musashi RNA binding protein 2 (*MSI2*) methylation and atopy.[29] Another large-scale EWAS with meta-analysis was performed by using a cross-sectional case-control design with

European children aged 4 to 16 years (the 4 cohorts: Barn/Children, Allergy, Milieu, Stockholm, Epidemiology [Sweden], Infancia y Medio Ambiente [Spain], Prevention and Incidence of Asthma and Mite Allergy [The Netherlands], and EDEN [France])] and found blood DNA hypomethylation of 14 CpG sites across childhood that were associated with asthma.[26] Transcriptional signatures associated with these sites demonstrated aberrant immune regulation, including activation of eosinophils, $CD8^+$ T cells, and natural killer cells. These epigenetic signatures were not present in cord blood cells at birth, so postnatal epigenetic remodeling related to disease pathology and/or environmental exposures likely occurred for these participants. This was also observed in a large, cross-sectional meta-analysis of blood DNA methylation in asthma in childhood.[27]

Regarding cord blood methylation signatures, in 1 EWAS, researchers investigated how DNA methylation affected risk of asthma in participants of the Infant Immune Study, which was an unselected birth cohort study in the United States. They found that methylation of the *SMAD3* gene at birth was associated with increased risk of developing childhood asthma.[30] Another EWAS, which also examined DNA methylation signals in cord blood cells, but in different cohorts, found 9 CpGs and 35 regions in newborns that were differentially methylated and associated with asthma development; these loci may represent predictive biomarkers of asthma risk by childhood.[27] These 2 studies indicate that DNA methylation patterns at certain loci present at birth are associated with asthma development later in life.

EWASs conducted with nasal epithelial cells from children have demonstrated that the nasal epigenome is perhaps a more sensitive biomarker for asthma and allergy. A cross-sectional EWAS of children from Project Viva in the United States found numerous differentially methylated CpGs and DMRs for asthma and allergy, including novel sites and replicated sites from studies in whole blood cells, as well as markers of lung function.[7] Analysis of DMRs was notable for lower DNA methylation levels of several CpGs corresponding to genes regulating eosinophilic activity (*EPX, CLC,* and *PRG2*) and the T_H2 cell response (*IL4* and *ZFPM1*). In this cohort, differential methylation of CpG sites and regions related to neutrophil degranulation and IL-4 and IL-13 signaling was associated with allergic asthma (vs no asthma diagnosis) and elevated fractional exhaled nitric oxide (vs normal levels). A different nasal epithelium EWAS in Dutch children with asthma and allergic rhinitis (the Prevention and Incidence of Asthma and Mite Allergy cohort) also found that IgE positivity contributed to differential DNA methylation signatures in patients with asthma, supporting a role for epigenetic alterations related to IgE sensitization in the pathogenesis and effects of atopic asthma and allergy.[31,32] However, the study found no significant CpGs for asthma alone, which the researchers suggested may have been due to low statistical power associated with low prevalence of asthma among the participants. Differential epigenetic signatures in nasal epithelial cells among children with asthma based on disease severity have been reported. In an EWAS involving 55 children of African American ancestry with asthma (the cohorts: Exposure Sibling Study and Genomics of Secondhand-Smoke Exposure in Pediatric Asthma Study in the United States), asthma severity was assessed by maximum respiratory symptom score; 816 differentially methylated CpGs and 10 regions were found to be associated with increased asthma severity.[25] The researchers also generated a receiver operating characteristic curve to predict asthma severity using 3 CpG sites (cg06654369, cg07463541, and cg1955158), which had a high area under the curve of 0.968. This demonstrates that these 3 sites could accurately predict asthma severity for children in the study. In addition to predicting disease severity, epigenetic markers in both the nasal epithelium and whole blood may also assess markers of respiratory lung function (eg, FEV_1) in children with asthma,[33] as well as clinical and complete remission of disease among children with asthma followed into adulthood.[34]

Although fewer studies have examined asthma in adults, 2 recent studies conducted with whole blood samples from adults provide new insights. In the Agricultural Lung Health Study, the largest EWAS of blood DNA methylation to be performed in adults with asthma, researchers classified 2286 participants (median age ranging from 60 to 63 years) based on asthma with atopy and asthma without atopy according to serum IgE levels.[35] In adjusted analyses, the researchers found 524 CpGs annotating to 382 novel genes for nonatopic asthma and 1086 CpGs annotating to 569 novel genes for atopic asthma, with 103 overlapping CpG sites. This indicates differential epigenetic signatures of atopy among patients with asthma.[36] Another study[24] included Taiwanese adults with asthma and chronic obstructive pulmonary disease, as these conditions can often overlap in adults,[37] and demonstrated that differential blood DNA methylation was associated with clinical phenotypes of disease status, such as airflow limitation, lung function decline, and disease exacerbations. Although the pathogenesis of chronic obstructive pulmonary disease differs from that of asthma, this study provides evidence that epigenetic markers may highlight risk of poor prognosis in patients with obstructive lung diseases.[38]

In summary, allergic diseases, including asthma, are associated with replicable DNA methylation patterns in blood and nasal cells. Very strong associations of atopy (presence of serum specific IgE to aeroallergens) and nasal DNA methylation have been observed.[7,32] Of interest, asthma and rhinitis have highly overlapping DNA methylation patterns in blood and nasal cells.[31,39] This supports the concept of shared mechanisms of different allergic diseases, as was previously suggested from large-scale genetic studies for asthma, rhinitis, and eczema.[40,41] Other allergic diseases, such as food allergy and eczema, have been studied less frequently, and well-powered studies are needed to address the epigenetic patterns of these diseases.

LINKING ENVIRONMENTAL EXPOSURES AND DNA METHYLATION TO ALLERGIC DISEASE

DNA methylation has been proposed to act as the interface between the environment and regulation of gene expression.[42] Environmental factors, particularly in the first years of life, could affect DNA methylation that extends across the lifespan (Fig 1). From conception to birth, the epigenome of developing tissues is reprogrammed, being rapidly erased from parental genomes and reestablished in the blastocyst stage and throughout cell differentiation and fetal development.[43] This represents a critical window for epigenetic reprogramming hypothesized to modify the risk of diseases later in life. Twin studies have indicated that the pattern of DNA methylation and familial correlation is stronger for CpG sites associated with early-life exposures and late-life health conditions.[44] Moreover, the developmental immunotoxicity of some compounds is hypothesized to occur via DNA methylation programming, altering cell differentiation and proliferation.[45] Some examples of genes that annotated to differentially

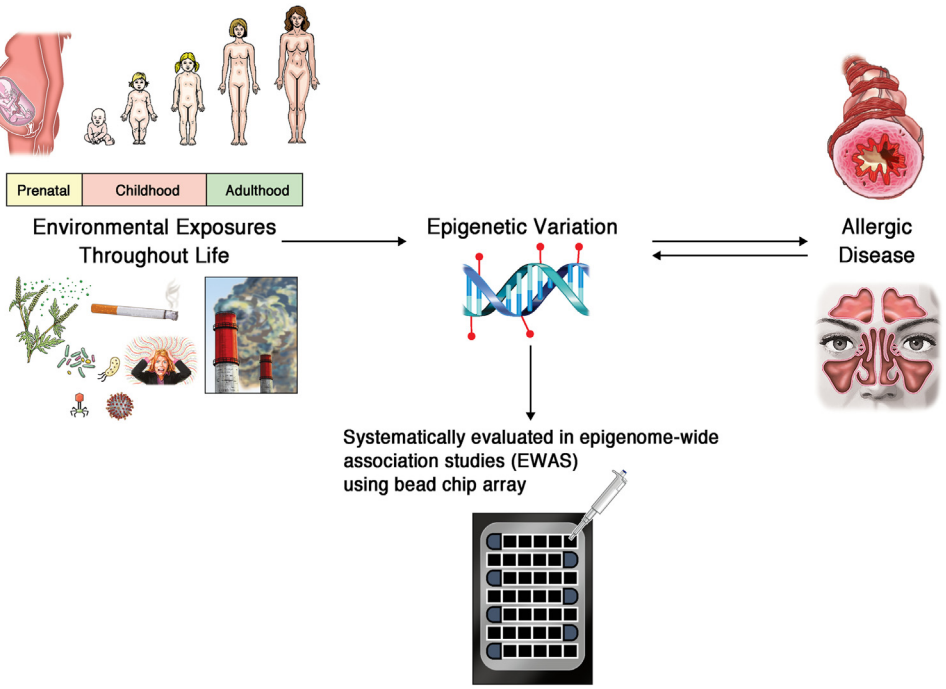

FIG 1. Interaction between environmental factors, the epigenome, and allergic disease. Environmental exposures, such as air pollution, cigarette smoke, allergens, social stressors, and microbes, which occur during the prenatal period as a fetus is developing as well as throughout childhood and adulthood, can affect the epigenome. As a result, the risk of allergic diseases may be increased throughout the life course. In EWASs, many DNA strands are obtained, often from mixed cell types such as blood or nasal cells and evaluated at hundreds of thousands of CpG sites by using bead chip arrays. DNA methylation is then quantified at a single-nucleotide resolution and expressed as a β-value, with a quantity between 0 and 1 representing the proportion of DNA molecules at which the single site is methylated.

methylated CpG sites in studies investigating DNA methylation alterations associated with environmental exposures, which may be related to allergic diseases, are listed in Fig 2 and described below.

Cigarette smoking is the best-characterized environmental exposure with robust DNA methylation associations. For example, maternal smoking *in utero* and active smoking in adulthood have profound effects on DNA methylation in cord blood and peripheral blood cells, respectively. The largest meta-analysis of the association between maternal smoking and cord blood DNA methylation showed that DNA methylation of 6,073 CpG sites in cord blood (false discovery rate [FDR] < 0.05) was associated with maternal smoking.[46] Importantly, there was evidence that most of these DNA methylation alterations persisted later in childhood, and genes relevant to asthma, such as *IL32* and *ESR1*, were differentially methylated relative to prenatal smoking.[46] Moreover, active smoking in adults had an even more striking association with blood DNA methylation: the largest-to-date meta-analysis of active smoking and DNA methylation alterations in adults identified 18,760 CpGs as being associated with smoking (FDR < 0.05), with many of these smoking-induced CpG methylation alterations persisting even after smoking cessation.[47] A systematic comparison of maternal smoking– and active smoking–related DNA methylation signatures showed that many CpG sites and pathways overlapped, but some CpGs, related to xenobiotic pathways were unique to maternal smoking.[48] Paternal tobacco smoke exposure has also been associated with increased DNA methylation of the immune-related genes *LMO2* and *IL10* in newborns and children aged 6 years, which was correlated with development of asthma.[49] The aryl hydrocarbon receptor repressor gene (*AHRR*), which is the top differentially methylated gene associated with both prenatal and adult smoking in leukocytes, has been shown to be involved in the relationship with asthma and lung function,[50,51] highlighting functional impacts on respiratory health.

Ambient air pollutants, such as fine particulate matter (particulate matter <2.5 μm in diameter [$PM_{2.5}$]) or diesel exhaust, are linked to increased asthma risk and exacerbation of respiratory symptoms. Several studies have tested associations between air pollutants and DNA methylation, mostly in blood cells. For example, a large meta-analysis of prenatal $PM_{2.5}$ and particulate matter less than 10 μm in diameter found associations with differential methylation of 14 and 6 CpGs in cord blood, respectively.[52] In this study, 2 differentially methylated genes, *FAM13A* and *NOTCH4*, were linked in genetic analyses to pulmonary function and asthma traits.[52] In nasal cells, exposure to $PM_{2.5}$ in the year before nasal sample collection was associated with DNA methylation of 362 CpGs (FDR < 0.05) and 10 DMRs of some genes that were previously associated with asthma in the same cohort.[53] In adults, there is evidence from controlled exposure experiments that short-term exposure to $PM_{2.5}$[54] and diesel exhaust[55] causally influences DNA methylation of leukocytes. In addition, crossover trials of target bronchial tissue have provided evidence of the effects of ozone[56] and diesel exhaust[57] on DNA methylation of respiratory target cells. In the diesel exhaust crossover trial, differential methylation of the *UNC45A* gene was reported; it has been associated with lung function among never-smokers, highlighting its functional implications.

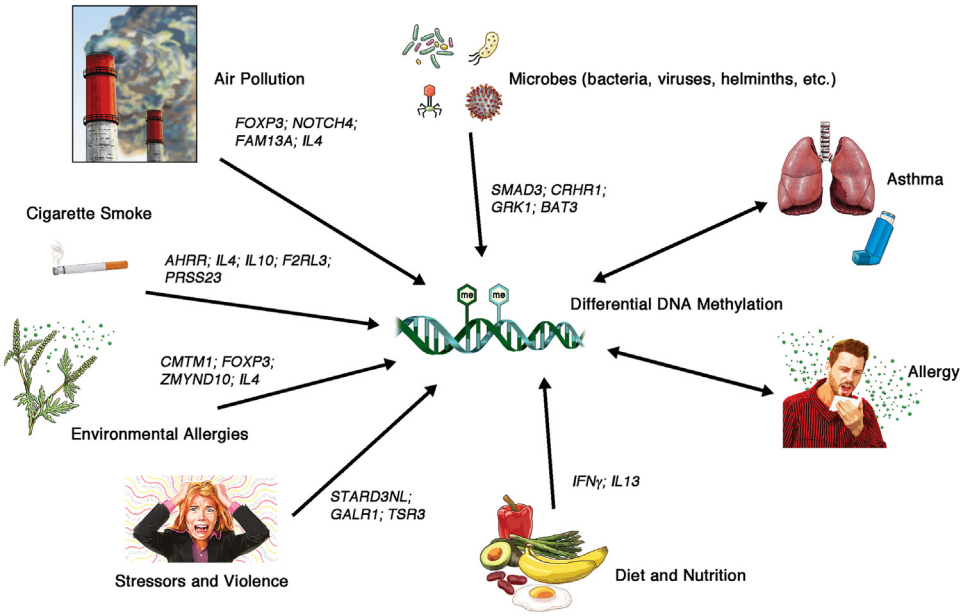

FIG 2. Selected examples of consistent gene annotations for differentially methylated CpGs associated with environmental exposures that may be related to allergic diseases.

Other important exposures related to asthma and allergy include stress and microbial exposures. Although EWASs on these exposures are sparse and mostly comprise small studies with candidate gene approaches, some important findings have emerged. For example, a novel study of airway microbial composition in early life demonstrated an association, with nasal epigenetic variation shown to partially mediate associations with allergic rhinitis risk.[58] Another study incorporated DNA methylation signatures associated with measures of exposure to violence and chronic stress, with some signatures also shown to be related to atopic asthma.[59] Other factors, such as breast-feeding, caesarean section, and diet, have not been well characterized with regard to their DNA methylation associations of different tissues and risk for allergic disease development.

RECENT EWASs ON THE ENVIRONMENT-ALLERGIC DISEASE RELATIONSHIP

There is a growing body of literature on how epigenetic modifications may mediate the relationship between a wide range of environmental exposures and asthma and allergy, although not all of the results are consistent.[2,60] New data on air pollutants show that they alter regional DNA methylation of *FOXP3* and *IL10*, which may increase risk of asthma.[61] In addition, environmental insults such as air pollution and allergens may work synergistically to affect the epigenome.[55] A recent EWAS of *Ascaris* roundworm infection (which is transmitted from contaminated soil and causes respiratory symptoms) involving 671 adult participants from the Respiratory Health in Northern Europe, Spain, and Australia study showed that *Ascaris* seropositivity is associated with differential methylation of genes, such as *CRHR1* and *GRK1*, which are linked to asthma pathogenesis.[62] In addition, an EWAS in children with asthma and/or allergic rhinitis found that 1 of the replicated CpG sites in the nasal epithelium (cg03565274) showed a positive association with pet exposure and that exposure to pets from birth onward is associated with hypermethylation at this site.[31] The evidence regarding the effects of early-life exposure to pets on immunologic sensitization and risk of asthma and allergy has been mixed.[63,64] In the previous study, no significant results were found for smoking, secondhand smoke exposure, or dampness and mold in the house. In a nasal epithelium EWAS involving 429 preterm infants followed to age 6 years, palivizumab administration for respiratory syncytial virus infection prophylaxis during infancy was related to global methylation differences but not to epigenetic markers of asthma at 6 years.[65] Social determinants of asthma outcomes have been studied previously,[66-69] but a recent study that examined epigenetic impacts was an EWAS of chronic stress and exposure to violence conducted with 487 children of Puerto Rican ancestry aged 9 to 20 years. In the meta-analysis of stress- or violence-associated CpG sites in nasal epithelium samples, researchers reported 12 differentially methylated CpG sites (annotating to the genes *STARD3NL, TSR3, CDC42SE2, KLHL25, GALR1, TMEM196, ANAPC13, SLC35F4, PLCB1, BUD13, OR2B3,* and *TEAD4*) associated with atopic asthma.[59]

INTERPRETATION OF EWASs ON ALLERGIC DISEASES

Overall, the EWASs discussed in the previous sections illustrate the complexity of interpreting cross-sectional studies of environmental exposures, disease, and DNA methylation. Longitudinal study designs are better suited to address any causal relationships between exposure, DNA methylation, and disease development.

In addition to environmental exposures, many other factors affect DNA methylation. They include genetics, age, cell type composition, subpopulations (in samples with cells of different

origin), and cell type activation (Fig 3). These are key factors that can contribute to variability in results and provide insights into disease development.

For example, the heritability of blood DNA methylation of CpG sites captured on the 450K array has been estimated as ranging from 3% to 20% and depends on the age of twins, with higher heritability estimates in adolescence and young adulthood than in middle age and birth.[44] This highlights the influence of the environment at specific life stages and the key role of genetic variation influencing DNA methylation variability. Large-scale meta-analyses have attempted to characterize how genetic variation affects blood DNA methylation; at specific loci, these are referred to as methylation quantitative trait loci. The largest study (32,851 participants) showed that genetic variants influence 45% of blood DNA methylation sites on the 450K array.[70] Genetic effects on DNA methylation were mostly polygenic (a median of 2 independent single-nucleotide polymorphisms [SNPs] affecting the same CpG site), explaining 15% to 17% of additive genetic variance of these DNA methylation sites. With a statistical technique called mendelian randomization, these data were used to disentangle cause and consequence of DNA methylation on disease risk. The SNPs that were selected are related to both 116 complex traits (including asthma and eczema) and DNA methylation. Interestingly, the researchers found very limited evidence that blood DNA methylation mediates the genetic effect on complex traits.[70] In contrast, in another study, cultured nasal epithelial cells exposed to rhinovirus were investigated for colocalization of asthma SNPs with those regulating gene expression and/or DNA methylation.[71] It was shown that among loci having an effect on nasal epithelial gene expression and/or DNA methylation, childhood onset asthma associated SNPs were significantly enriched—more strongly so than adult-onset asthma SNPs. Moreover, 24 SNPs showed evidence of colocalization of asthma, regulation of mRNA, and DNA methylation. Causal modeling showed that in these cases, DNA methylation likely mediated the effects of the SNPs on gene expression.[71] Thus, DNA methylation may likely be implicated in the causal pathway of many asthma SNPs that regulate gene expression in nasal epithelial cells, which was also previously observed in bronchial epithelial cells.[72,73]

HETEROGENEITY AMONG CELL AND TISSUE TYPES

EWASs of DNA collected in samples with mixed cells might have findings that reflect underlying cell type composition, cell type activation, or a combination of both (Fig 3). Interpretation of the first large EWAS on asthma showed that the asthma-associated differentially methylated sites in whole blood were partly driven by cell type (blood eosinophilia).[26] Subsequent analysis in sorted eosinophils showed that the numbers of these CpGs were markedly lower in eosinophils from patients with asthma than in those from healthy controls, suggesting changes within target cell types. The study's authors reported that blood DNA methylation in asthma may serve as a marker of activation and influx of blood eosinophils.[26]

From the current evidence generated in samples from different cell sources, it has become evident that DNA methylation is a strong marker of cell type and cell activation. However, as most EWASs survey mixtures of cell types (ie, DNA isolated from whole blood or nasal brushing), it is not possible to address cell type epigenetic signatures from cell type activation events. Follow-up functional and experimental validation could yield promising targets or leads for biomarker development.

For example, blood DNA methylation patterns in asthma and allergy reflect eosinophilic inflammation,[26,27] and DNA methylation in nasal cells in allergy reflects an influx of immune cells, such as T cells, and epithelial changes.[32,39] Interestingly, a recent multiomic analysis of the diagnostic properties of SNP-panels, DNA methylation in blood and nasal cells, and personal and environmental factors for childhood allergy showed that DNA methylation in nasal cells is by far the strongest predictor of allergy and that SNP panels and personal and environmental factors did not contribute to disease prediction. In this study, nasal DNA methylation at only 3 CpG sites could adequately classify allergy in childhood.[74] When the 3 nasal CpG sites were linked to nasal gene expression, it was shown that gene transcripts associated with the 3 CpG sites were characteristic of inflammatory cells, such as T cells and monocytes. Therefore, the authors concluded that DNA methylation was able to capture the influx of inflammatory cells that enter the nasal mucosa in allergic conditions.

EPIGENETIC AGING IN ALLERGY AND ASTHMA

Methylation of DNA has been shown to vary strongly with age, which has yielded novel epigenetic aging biomarkers shown to be highly accurate predictors of chronologic age, morbidity, and mortality. Data on DNA methylation at specific CpG sites are input into epigenetic clock calculators to determine an "epigenetic age" for each study participant; this number is then compared with each participant's chronologic age. This summary measure derived from EWAS array data can be used as a proxy for biologic aging. Since the deviation from chronologic age, or epigenetic age acceleration (EAA), has been associated with asthma and allergy, we have briefly highlighted emerging studies on these promising biomarkers. In a cohort of children, leukocyte EAA was cross-sectionally associated with higher serum IgE levels and greater odds of atopic sensitization.[75] In addition, studies of EAA in nasal cells showed the greatest increase in EAA in patients with allergic asthma.[7] However, these studies were cross-sectional, and longitudinal data collected at multiple time points are needed to determine whether EAA predicts disease onset, severity, or progression. A small case study reported that EAA is associated with asthma response to oral steroids in a sex-specific manner.[76] Future studies should evaluate whether epigenetic aging biomarkers and acceleration can predict, classify, and target treatments of asthma and allergy as well as address causal relationships.

CURRENT CHALLENGES AND FUTURE IMPLICATIONS

DNA methylation captures many factors relevant to allergy and asthma. However, how much of the variance of DNA methylation is explained by environmental factors, genetics, age, sex, and cell type composition and activation is unknown. This may be dependent on specific CpG sites or genomic regions, life stage, and cell or tissue type. Many environmental exposures are related to DNA methylation, but few examples of how these findings directly and causally relate to allergy or asthma have been published to date. Because it is difficult to infer causality from

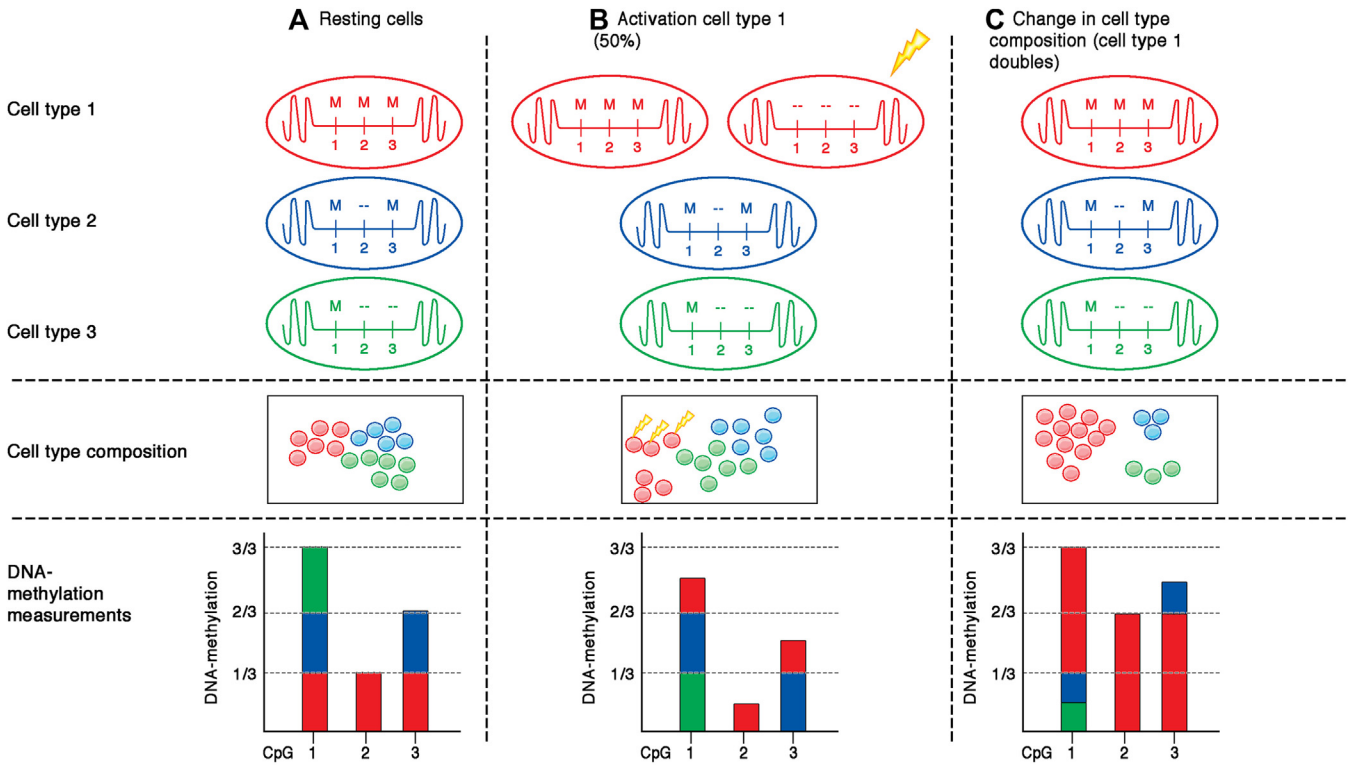

FIG 3. Interpretation of differentially methylated positions in EWASs in mixed cell samples. DNA methylation is measured at 3 CpG sites in a mixed cell population of 3 cell types. **A,** The contribution of each cell in a resting condition to total DNA methylation at this site. **B,** The scenario in which 50% of the red cell type is activated, as shown by demethylation at the 3 CpG sites and, consequently, lower total DNA methylation at all 3 CpG sites. **C,** The effects of an increased influx of the red cell type on total CpG methylation.

cross-sectional studies, we recommend that future prospective studies investigate the environment and DNA methylation before disease onset to shed more light on the topic.

An important challenge in current epigenetic studies is the annotation of the differentially methylated CpGs and genomic regions. Annotation by location assumes that differential CpG methylation affects the function of the closest gene. However, for many CpG sites, this has not been shown. For example, among nasal CpG sites related to atopic asthma, only 429 (6.1%) were associated with the gene expression of a nearby gene.[77] Functional interpretation of CpG methylation by matched gene expression is therefore needed to better infer the biologic relevance of epigenetic findings. Notably, this is further improved when studying a single cell type rather than bulk samples consisting of different cell types. Development of single-cell epigenetic and transcriptomic methods could further improve our insight into the complex regulation of gene transcription by exogenous factors.

Another challenge is the genomic coverage of the currently available arrays; for example, the 450K array covers approximately 1.7% of the estimated 28 million sites of the DNA methylome.[78] Moreover, investigators are dependent on the design of the array, with EPIC arrays having incomplete coverage of *FANTOM5* enhancers (58%) and even lower coverage of proximal and distal regulatory elements. Alternatively, DNA methylation can also be measured by using next-generation sequencing, which has substantially improved coverage of the genome but still comes at significant costs.[10] The development of custom arrays of high-value CpGs for allergic sensitization in nasal epithelial cells is a potentially promising solution.[79] As costs decrease, we expect that the field will continue to grow and provide useful clinical markers.

On the basis of the current evidence, future applications of DNA methylation can be foreseen. An existing clinical application of DNA methylation is a US Food and Drug Administration–approved DNA methylation panel for colorectal cancer screening.[80] In the field of allergy, several applications can be envisioned. Nasal methylation panels could serve as a less invasive marker of allergy, specifically, in young children in whom it is difficult to diagnose asthma or allergic rhinitis, or to predict treatment responsiveness based on epigenetic aging of target tissue, as has been preliminarily shown.[76] DNA methylation could also be used to identify novel drug targets, provided DNA methylation panels that reflect pathogenic mechanisms are included. Indeed, analysis of the integration of disease-associated SNPs and DNA methylation with gene expression in epithelial cells has shown examples of how disease SNP associations with gene expression are mediated by DNA methylation. Future work that includes causal modeling could assist in selecting the correct drug target. Alternatively, it is possible that DNA methylation may be a consequence of disease and thus not a proper drug target but rather a biomarker of disease progression, treatment effectiveness, or specific clinical endotypes. Furthermore, DNA methylation could also be thought of as an environmental sensor. For instance, given the strong, consistent association between DNA methylation at the *AHRR* locus and smoking, DNA methylation has been proposed as a marker of smoke exposure that is potentially even better

than measuring urine cotinine levels. Finally, DNA methylation is strongly affected by inhaled corticosteroids, and we propose that measurement of DNA methylation at certain CpG sites, such as the *FKBP5* locus,[81] could serve as a marker of actual steroid use by patients in order to measure medication adherence.

In summary, EWASs have highlighted distinct DNA methylation patterns associated with allergy and asthma and how some environmental factors can affect DNA methylation across different cells and tissues. In blood and nasal cells, EWASs of allergic disease have identified replicable sites of differential DNA methylation,[7,26,27] although these have largely been limited to cross-sectional rather than predictive, longitudinal associations. Interpretation of DNA methylation in mixed cell populations shows evidence of allergy-associated patterns of influx of immune cells as well as cell type activation. Current evidence supporting a causal role of DNA methylation in allergy is limited, and prospective studies that enable a better interpretation of the cause-effect relationship of DNA methylation and allergy are much needed. We envisage the first clinical application of DNA methylation as a diagnostic biomarker or a marker that may assist in personalized medicine approaches in eosinophilic asthma and serve as a measure of epigenetic aging of target tissue such as nasal cells to monitor disease progression. Future studies that combine whole genome sequencing and single-cell methods will undoubtedly shed more light on the pivotal role of DNA methylation in allergic disease.

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