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The Environment, the Epigenome, and Asthma

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Abstract

Asthma prevalence has been on the increase, especially in North America compared to other continents. However, the prevalence of asthma differs worldwide and in many countries the prevalence of asthma is stable or decreasing. This highlights the influence of environmental exposures, such as allergens, air pollution, and environmental microbiome, on disease etiology and pathogenesis. The epigenome may provide the unifying mechanism that translates the influence of environmental exposures to changes in gene expression, respiratory epithelial function, and immune cell skewing that are hallmarks of asthma. In this review, we will introduce the concept of the environmental epigenome in asthma, summarize previous publications of relevance to this field, and discuss future directions.

Keywords

asthma; DNA methylation; epigenetics; nasal epithelium; microbiome; air pollution; allergens

Asthma remains an important public health problem in the United States and worldwide because of high morbidity and inadequate disease control. Recent data from the International Study of Asthma and Allergies in Childhood (ISAAC) demonstrate geographical differences in asthma prevalence, suggesting that the environment and epigenetics play a key role in this disease. [1] While asthma is more problematic in children, this disease also affects the health of adults. The dynamic and unique biological responses that are triggered by allergens and air pollutants have proven difficult to predict and prevent. This review will focus on the epigenetic mechanisms that regulate the response to environmental exposures and are critical to primary and secondary prevention of asthma.

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Etiology of asthma

While inheritance, [2–5] parent-of-origin, [6–8] general environment, [9–17] immunization,[18] *in utero* exposures, [19–24] and Th_2 immunity [25] play important roles in the etiology of asthma, there is no unifying mechanism accounting for these etiologic events.

Asthma concordance in MZ twins is only ~50%, [26] and the heritablitity of this disease is 0.40–0.85. [27, 28] Initial genome Wide Association Studies (GWAS) identified three putative susceptibility loci (5q22.1 near *TMEM232*, 11q13.5 near *C11orf30*, and the HLA region) associated with asthma risk, [3, 29] but the scope of these studies was limited. Moffatt *et al.* reported that genetic variants of *ORMDL3* contribute to the risk of childhood asthma. [30] Recently, meta-analysis of GWAS identified seven asthma genetic risk loci (*HLA-DQ*, *IL33*, *ORMDL3/GSDMB*, *IL1RL1/IL18R1*, *IL2RB*, *SMAD3*, and *TSLP* [31, 32]) and ten loci near *TLR6*, C11orf30, *STAT6*, *SLC25A46*, *HLA-DQB1*, *IL1RL1*, *LPP*, *MYC*, *IL-2*, and *HLA-B* that influence allergic sensitization. [33] In aggregate, approximately 50 replicated genes have been identified from association studies, several genes by linkage and fine mapping, and one gene identified by GWAS. However, the effect estimates are modest (odds ratios between 0.5 and 1.5), and it has been estimated that these gene variants predict less than 10% of the heritability of asthma. [34]

Allergens and Asthma

Allergic sensitization is a critical risk factor for childhood asthma, conferring a 4–20 fold increase in the risk of developing disease. [10, 35] Both indoor (molds, house dust mites, cockroaches, rodents, and pets) and outdoor (pollens from trees, grass, and weeds) allergens are critical environmental triggers of asthma. [36–38] Seminal studies in this field have clearly demonstrated a role of the environment in the development of asthma; exposure to house dust mite, cat and dog allergen early in life and continuing throughout childhood determined the course of chronic airway hyper-responsiveness and impairment of lung function, [35] sensitization to dog, cat, or *Alternaria* was associated with increased bronchial responsiveness in children with mild to moderate asthma, [39] children who were both allergic to cockroach and exposed to high levels of this allergen had significantly more asthma-related hospitalizations, days of wheezing, missed school days, and nights with lost sleep, [40] and mouse sensitization/exposure was associated with acute care visits, decreased lung function, fraction of exhaled nitric oxide levels, and bronchodilator reversibility. [41] Pet allergens may also be protective in some settings although the evidence for this is mixed. [36, 42]

Air pollution and Microbial Factors and the Severity of Asthma

Air pollutants are known to exacerbate the symptoms of asthma and may also play a role in the initiation of this disease. [43–45] In most urban areas, and increasingly suburban areas, components of traffic-related emissions are a major source of air pollution. However, air pollution represents a complex exposure with inorganic and organic components. Particulate matter (PM) carries both environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs) formed during the incomplete combustion of fossil fuels and oil products, [46] and

immune stimulating agents such as pollens, endotoxin and fungal spores. PAHs are widespread environmental contaminants formed as a result of incomplete combustion of organic materials and are a particularly toxic component of air pollution. [47, 48] PAHs in PM such as phenanthrene or other components of diesel exhaust can also directly enhance IgE production *in vitro.* [49] Endotoxin, a structural component of membranes of Gram negative bacteria, is ubiquitous in urban and suburban environments both in homes/daycares/schools [50–53] and is a component of air pollution. [54, 55] In addition to endotoxin, children who grow up in rural and inner city areas are exposed to many other aerosolized microorganisms and their toxins. [56, 57] Interestingly, germ-free (GF) mice are more prone to a Th_2 phenotype and allergic airway disease, and this risk can be reduced by exposure to a diverse microbial flora. [58] Moreover, mono-colonization with a common intestinal bacterium *Bacteroides fragilis* restores Th_2/Th_1 balance using a mechanism dependent on stimulation of Th_1 CD4+ T cell proliferation by a zwitterionic (neutral molecule with positive and negative charges) coat polysaccharide (ZPS) that directly activates CD4+ T cells, [59, 60] and can also be potent T_{reg} cell inducers. [61] ZPSs have been characterized in commensals from multiple body sites including *Streptococcus pneumoniae* in the upper respiratory tract, and ZPSs from different organisms have similar biological properties and can display immune cross-reactivity. [59, 62, 63] Interestingly, the prevalence of *Staphylococcus aureus* (which produce ZPS and is present in bedding/household dust) was inversely associated with asthma. [64, 65]

Introduction to Epigenetics

Epigenetic mechanisms control expression levels of genes without changing DNA sequence. Hypermethylation of cytosines within CpG islands in gene promoters leads to gene silencing and hypomethylation leads to active transcription. [66, 67] More recent studies have demonstrated that methylation of less CpG dense regions near islands ('shores')[6869] and within gene bodies [70, 71] is also important in regulation of gene expression and alternative splicing, and that the relationship between methylation and transcription is more complex. Further adding to this complexity is the presence of methylation marks in non-CpG context [72, 73] and presence of 5-hydroxymethylcytosine, which may be a mark of demethylation.[74] Finally, the most recent data from international consortia (ENCODE[75], FANTOM5[76], and Roadmap Epigenomics[77]) identify enhancers as critical regions involved in regulation of gene expression in addition to promoters. Promoters and enhancers are also characterized by the presence of a number of histone modifications, with histone methylation and acetylation being the most common.[78, 79] Acetylation of lysine 27 on the histone H3 (H3K27ac) is one of the most informative single histone modifications. It is known to mark active enhancers and is also enriched at active promoters. Among histone methylation marks, H3K4me1 is present at poised and active enhancers while H3K4me3 marks poised or active promoters. Although non-coding RNAs such as micro RNAs (miRNAs) and long intergenic noncoding RNAs (lncRNAs) are sometimes viewed as a part of the epigenome as they are involved in regulation of gene expression,[80] they will not be discussed in the current review and are reviewed elsewhere.[81]

Environment and the Epigenome

While some of the epigenetic marks are heritable (such as imprinted loci[82], for example) and genome-wide studies demonstrate a genetic component to inter-individual variation in DNA methylation [83–86] and histone modification profiles, [83–85] epigenetic marks are also strongly influenced by the environment.[86] Epigenetic processes translate environmental exposures associated with disease risk into regulation of chromatin, which shapes the identity, gene expression profile, and activity of specific cell types that participate in disease pathophysiology.[87] Both DNA methylation and histone modifications are mutable and dynamic, responding to the environment, disease and aging.[67, 86, 88] These alterations may persist for the life of a cell, demonstrate transgenerational inheritance, or may be altered prenatally and postnatally, influencing gene expression differentially throughout life.[89, 90] We have previously proposed that epigenetic marks may be the missing link that connects environmental exposures in genetically predisposed individuals to transcriptional changes associated with development of asthma.[91]

Asthma Epigenetics

Epigenetic mechanisms, as a cause of asthma,[91] build on our current knowledge about the etiology of asthma: non-Mendelian[3] and parent-of-origin inheritance,[6] influence of direct[9] and *in utero*[92] exposures, and a strong component of immune regulation.[25] Our early work in mice showed that *in utero* exposure to high methyl donor diet resulted in an increase in airway inflammation (eosinophil recruitment and concentrations of IL-4 and IL-13), increase in serum IgE, a skewing of the lymphocytes toward a Th_2 phenotype, and hypermethylation of *Runx3*, a transcription factor involved in regulation of Th_2 immunity (unpublished data). It is well established that epigenetic mechanisms regulate expression of transcription factors and cytokines important in T cell differentiation (Th_1, Th_2, and T_{regs}).[93–100] Another animal study contributed to our knowledge of the critical role of DNA methylation in allergic airway disease by showing that global DNA demethylation agent 5-aza-2′-deoxycytidine (5-AZA) prevented Th2 skewing and rebalanced Th1/Th2, and used adoptive transfer experiments to demonstrate that 5-AZA treated CD4+ T cells protect against allergic airway disease.[101]

Early studies in human cohorts demonstrated an association of DNA methylation in a few candidate genes in peripheral blood[102], and buccal[103, 104] and nasal[105] cells with asthma phenotypes but did not elucidate the role of DNA methylation in the control of gene expression. Our work in African American inner city children identified 81 differentially methylated regions (DMRs) in peripheral blood mononuclear cells (PBMCs) associated with allergic asthma.[106] Methylation changes in PBMCs are modest (median 1.3%; range 0.02%–3.1%) but consistent with the majority of DMRs hypomethylated in asthma. Several immune genes were hypomethylated in asthma, including *IL-13*, *RUNX3*, and *TIGIT*. Hypo- and hypermethylated genes were associated with increased and decreased gene expression respectively ($P<0.6\times10^{-11}$). We further explored the relationship between DNA methylation and gene expression using an integrative analysis and identified additional candidates relevant to asthma (*IL-4* and *ST2*). Our group also contributed to a study that identified replicated associations, in three independent cohorts, between IgE and low methylation at 36

loci.[107] Genes annotated to these loci encode known eosinophil products, and also implicate phospholipid inflammatory mediators, specific transcription factors, and mitochondrial proteins. We confirmed that methylation at these loci differed significantly in isolated eosinophils from subjects with and without high IgE levels. The top three loci accounted for 13.5% of IgE variation, explaining 10 fold higher variance than that derived from large genome-wide association studies (GWAS). A recent publication by another group identified asthma-specific enhancers in primary CD4+ T cells, marked by gaining the histone H3K4me2 mark during Th_2 cell development.[108]

In the nasal epithelia of inner city African American children, we identified substantial (median 9.5%, range: 2.6–29.5% methylation change) methylation changes, both in the form of single CpG methylation (differentially methylated probes[DMPs]) and regions (DMRs) that are associated with their disease and changes in gene expression.[109] The magnitude of the methylation changes observed in nasal epithelia of these asthmatic children approaches that reported in malignancies.[110, 111] 60% of genes that are differentially expressed in the asthmatic nasal epithelium have significant associations between DNA methylation and gene expression; these include asthma genes (*ALOX15*, *CAPN14*, *POSTN*), genes involved in inflammation and immunity, cell adhesion, extracellular matrix, obesity and autophagy, and epigenetic regulators, among others. 30% of the genes we identified were also found in an IL-13 DNA methylome signature of cultured airway epithelial cells of asthmatics, additionally demonstrating the relevance of our findings to allergic asthma. Collectively, these studies underscore the importance of the epigenome as a modifier of transcriptional profiles associated with asthma and demonstrate the potential utility of profiling nasal epithelia in the context of studying environmental exposures in asthma.

Nasal Epithelium as a Biosensor of the Environment with Relevance to the Disease Process

The nasal and airway epithelium is the primary interface with the respirable environment, interacts with air pollution,[43] allergens[112–114] and other environmental stimuli,[115] and directs airway inflammatory, immune, and regenerative responses to these exposures. Gene expression profiles of the asthmatic airway epithelium have identified genes associated with exposure to endotoxin,[116] house dust mite allergen,[116] cigarette smoke,[117] asthma,[117, 118] and disease subtypes.[119] Importantly, it has been shown that the nasal epithelium is a reasonable proxy for the airway epithelium; nasal airway transcriptome mirrors the bronchial airway transcriptome and reflects asthma status as well as Th_2-related subphenotypes of disease.[120] Our more recent work demonstrates that nasal epithelia capture disease activity seen in the lung airway epithelia but that there are many more significant associated DNA methylation changes in the nasal epithelia (Figure 1), suggesting an important role for the environment in influencing these epigenetic changes[121] and the need to understand environmental exposures that are driving these changes.[122] While the solubility and particle size of ambient air pollutants[123] and allergens[114] are key determinants of deposition and response, the nasal epithelium is the most proximal portion of the airway and may represent the only portion of the airway that comes in contact with relevant components of the environment that trigger airway responses. As such, nasal epithelium is

constantly exposed to ozone, endotoxin, allergens, and other toxins from air pollution, functions to filter air pollution particles by the process of mucociliary clearance, and is an active component of both the immune and respiratory systems.

Epigenetic Changes Associated with Exposure to Air Pollution

Air pollution influences the peripheral blood epigenome among adults.[124–135] These studies have shown that both short[125, 127, 133] and long[126, 129] term exposures to PM impact DNA methylation, and includes genes in innate immunity (*TLR4, TLR2*)[125, 136] and asthma (*HLA-DOB, HLA-DPA1, CCL11, CD40LG, ECP, FCER1A, FCER1G, IL9, IL10, IL13, MBP*).[129] One study has also demonstrated the effect of PM_{10} and PM_{BC} on 5-hydroxymethylcytosine[134]. Similarly, cigarette smoke exposure has profound influence on DNA methylation among adult smokers[137, 138] and as second hand smoke exposure (ETS) in childhood[139, 140] and *in utero,*[141–143] and also affects innate immune genes (*CD14*).[139] Diesel exhaust particle exposure (DEP) has been associated with DNA methylation changes in peripheral blood[144] and lung epithelial cells.[145] DNA methylation in adults[146] and children both as a result of direct[147] and *in utero*[148, 149] exposure is also influenced by PAHs, a bi-product of incomplete combustion of organic materials in airborne pollution. In children, PAH exposure has been associated with increased methylation of *IFNG*[148] and the T_{reg} transcription factor *FOXP3* as well as impaired T_{reg} function.[147]

Epigenetic Changes Associated with Exposure and Sensitization to Allergens

Very few studies to date have examined the relationship of allergen exposure and sensitization to changes in epigenetic marks. Sensitization to several allergens (tree, grass, house dust mite, and ragweed) has been associated with changes in DNA methylation in peripheral blood of older adults[150] and in bronchial epithelial cells of adults in controlled exposure settings.[145] CD4+ T cells isolated from *ex vivo* grass pollen extract stimulated PBMCs of patients with seasonal allergic rhinitis have extensive DNA methylation and gene expression changes compared to control patients.[151] Decreased DNA methylation in the *CD14* promoter has been associated with increased CD14 expression in placentas of mothers living on a farm compared with mothers not living on a farm [152] and methyl-CpG-binding protein Mbd2 has been shown to control Th2 induction by dendritic cells.[153] No studies have been performed on specific indoor allergens.

A few studies have begun to understand the complex relationship of multiple exposures. DEP exposure in combination with allergen resulted in hypermethylation of the Th_1 cytokine *Ifng* and hypomethylation of the Th_2 cytokine *Il4* in mice.[154] In human airway epithelial cells, controlled exposure to allergen alone, diesel exhaust alone, or allergen and diesel exhaust together (coexposure) led to significant changes in only 7 CpG sites genome-wide at 48 hours.[145] However, when the same lung was exposed to allergen and diesel exhaust but separated by approximately 4 weeks, significant changes in more than 500 sites were observed. These findings suggest that specific exposures can prime the lung for changes in DNA methylation induced by a subsequent insult.

Epigenetic changes and the Microbiome

The microbiome represents the multitude of microbes (bacteria, archaea, microbial eukaryotes such as fungi, and viruses) that live in the environment and that also inhabit our bodies. Exposure to a greater diversity or unique repertoire of microbes via bedding or household dust, [64, 15565] birth by vaginal rather than cesarean section,[156] or relatively restricted exposure to antibiotics in early life[157–159] have all been associated with decreased incidence of childhood asthma in epidemiological studies. Furthermore, germ-free (GF) mice (i.e. mice born and raised in sterile isolators devoid of microbes) have increased airway resistance, increased total bronchoalveolar lavage fluid cell numbers, eosinophilia, and proinflammatory cytokine production, and higher serum IgE levels compared with conventional specific-pathogen-free (SPF) mice in an ovalbumin (OVA)–driven allergic-asthma mouse model.[160] The relationship between microbial exposures and asthma susceptibility is particularly strong in early life. For instance, in humans, the largest reduction in risk of developing respiratory allergies with exposure to a farming environment is seen among those who are exposed prenatally and continuously thereafter.[155] Furthermore, colonization of neonatal but not adult GF mice with a conventional microbiota protected them from OVA-driven allergic asthma.[160]

The mechanisms that mediate the influence of early-life microbial exposures on asthma/immune phenotypes are largely not understood, but microbially-mediated modification to the epigenome may in some cases provide this “missing link.” We are only beginning to understand the extent to which the microbiome exerts influence on host gene expression via modification to the epigenome. Different gut (fecal) microbiota compositions in humans have been associated with distinctive DNA methylomes in blood. [161] Experiments with GF mice have also indicated a link; for instance the methylation level of the TLR4 gene was significantly lower in Intestinal Epithelial Cells (IECs) of the large intestine of GF compared to conventional mice.[162] Finally, *in vitro* experiments have allowed for exploration of how different microbes may affect the epigenome; immature enterocytes exposed to probiotic and pathogenic bacteria showed over 200 regions of differential DNA methylation, with decreased DNA methylation of genes associated with cytoskeleton/actin remodeling and cell adhesion functions after exposure to pathogenic gram-negative bacteria (*Klebsiella spp.*).[163] Interestingly, fetal epithelial cells were more sensitive to these pathogenic-specific changes than adult epithelial cells.[163]

The mechanisms by which specific microbes may affect the epigenome are not well known but include via their metabolic activity. For instance, the short-chain-fatty-acid (SCFA) butyrate, a key product of the microbial fermentation of dietary fiber in the gut, induces the differentiation of colonic T_{reg} cells and this was associated with enhanced histone H3 acetylation in the promoter and conserved non-coding sequence regions of the *Foxp3* locus.[164] It has also been proposed that microbiota can exert influence by altering the availability of chemical donors for DNA or histone modifications,[165] for instance various probiotic bacteria have the ability to produce/consume folate and can affect both plasma and fecal folate levels following oral consumption.[166] The relationship between the epigenome and microbiome can also “go both ways”, with differential epigenetic marks also affecting which microbes colonize hosts; for instance, epigenetic alterations induced by treating

pregnant mice with dexamethasone were associated with altered composition of the gut microbiome of their offspring.[163]

Supporting a specific importance for epigenome modifications in mediating early microbe exposures and development of asthma, one study showed epigenome modification as a driver of the protection conferred by early colonization of GF mice in an OVA–driven allergic-asthma mouse model.[160] Specifically, the accumulation of invariant natural killer T (iNKT) cells in the colonic lamina propria and lung in 8 week old GF mice, [160] was linked to increased expression of the mRNA for CXCL16, a ligand of chemokine receptor CXCR6 on iNKT cells. A region 5′ of the *Cxcl16* gene that contains five potential CpG sites was hypermethylated in the colon and lungs, but not in other tissues such as the spleen and liver, in GF compared to SPF conditions. Since this observation was made by comparing GF mice to those colonized with a diverse microbiota, the specific bacteria and molecular factors that mediate this epigenetic modification remain to be discovered.

The complex interplay between early life microbial exposures and those that occur over time in the state of the epigenome and development of asthma susceptibility/exacerbations is not well understood. Given the high magnitude of methylation changes in genes differentially expressed in the asthmatic nasal epithelium, [109] it is interesting to consider that the nasal cavity is home to a complex and poorly understood community of microorganisms. Since mucous in the nasal cavity captures PM, some component on the nasal microbiome certainly represents the “respirable” microbiome (i.e. microbes that have been deposited on inhaled particles); the nasal microbiome thus can in part can be considered to be an “exposure” and in part a community that have adapted to inhabit and in some cases closely interact with the host.

Prior culture-independent studies of nasal microbiome have focused on bacteria in the nasal vestibule (e.g. the anterior nares) and nasal cavity, and have found the genera Corynebacterium, Propionibacterium, and Staphylococcus (all common skin bacteria) to be among the most frequent colonizers[167, 168] with a relatively low prevalence of Propionibacterium that can use sebum as a growth substrate in the nasal cavity compared to vestibule.[167] Specific microbial taxa[169] and their expressed genes[170] in the nasal cavity differ significantly between asthmatics and controls. Asthma-associated nasal microbes are also associated with differential expression of host genes such as increased expression of mediators of inflammation[169, 170] and apoptosis.[169]

Future Perspective

A major goal of this field is to understand the complex interaction of the microbiome, indoor allergens, and air pollution with the dynamic biological responses in the nares that predispose toward a Th_2 phenotype and place individuals at risk of asthma (Figure 2). These environmental factors are likely to influence the epigenome differently based on genetic variants of the host. Isolated studies focused on genetics, epigenetics, pathobiology, and the microbiota have so far provided only a partial picture regarding disease risk and its mechanistic basis. A comprehensive and integrated approach to asthma will: 1) establish the basic molecular profiles to develop novel molecular insights into disease etiology and

clinical severity/extent; 2) produce environmental and biological signatures that will create a roadmap for primary and secondary prevention of asthma; and 3) provide the rationale and targets/biomarkers for intervention in this disease that remains a significant public health problem, especially among children. While other tissues/cell types may be valuable for studies focused on immunology of asthma, the nasal epithelium is the ideal surrogate tissue for studies of environmental asthma as it is easily accessible in children and adults, has substantial epigenetic and gene expression changes associated with asthma, and appears to be an excellent proxy for the bronchial epithelium.

The integrative approach would require the use of systems biology to understand how different elements of the environment, in the context of genetic susceptibility, interact to lead to changes in the epigenome and transcriptome, and ultimately to Th_2 cell skewing and specific disease phenotypes. Systems biology generally refers to a process of identifying networks of molecular pathways based on the evidence from multiple datasets and has been reviewed in the context of asthma.[171]

Findings from integrative studies will identify elements of exposures that have the most prominent effect on the epigenome/transcriptome. It is likely that exposures will be different depending on the geographical region but also that the host will respond differently to them based on their genetics and epigenetics, suggesting that personalized intervention will be needed, which aligns well with the goals of personalized or precision medicine.[172] Importantly, we view epigenetic marks influenced by exposures as biomarkers of disease that would allow us to treat patients early in the course of the disease but may also provide specific treatments for different asthma endotypes[173] and difficult-to-control asthma.[174] Developing a greater understanding of how exposures to modifiable factors such as the microbiome may dynamically affect the epigenome of key immune and or asthma-associated genes may pave to way to novel intervention strategies.

These approaches will also prioritize genes that have epigenetic marks influenced by multiple exposures as the most relevant candidates for therapeutic intervention. DNA methylation changes have been shown to drive tumor formation and malignant progression,[175] and as such have established basic mechanisms for disease pathogenesis and targets for intervention in cancer. DNA methyltransferase (DNMT) inhibitors have been approved for the treatment of myelodysplastic syndrome[176, 177] and are in clinical trials for treatment of solid tumors.[178, 179] While currently available DNMT inhibitors lack specificity for gene(s) of interest, locus-specific therapies are currently being developed.[175, 180]

Our ability to predict, prevent, and control asthma will be substantially advanced by understanding the complex interactive relationships of the microbiome, indoor allergens, and air pollutants with the dynamic biological responses in the airways that predispose toward a Th_2 phenotype.

Abbreviations

5-AZA	5-aza-2′-deoxycytidine
CpG	cytosine followed by guanine in DNA sequence

DEP	diesel exhaust particle
DMP	differentially methylated probe or position
DMR	differentially methylated region
DNA	deoxyribonucleic acid
DNMT	DNA methyltransferase
GF	germ free
GWAS	genomewide association
IEC	intestinal epithelial cells
iNTK	invariant natural killer
miRNA	micro RNA
ncRNA	non-coding RNA
OVA	ovalbumin
PAH	poluaromatic hydrocarbon
PBMC	peripheral blood mononuclear cells
PM	particulate matter
RNA	ribonucleic acid
SCFA	short-chain-fatty-acid
SNP	single nucleotide polymorphism
SPF	specific pathogen free
Th	T helper cells
ZPS	zwitterionic coat polysaccharide

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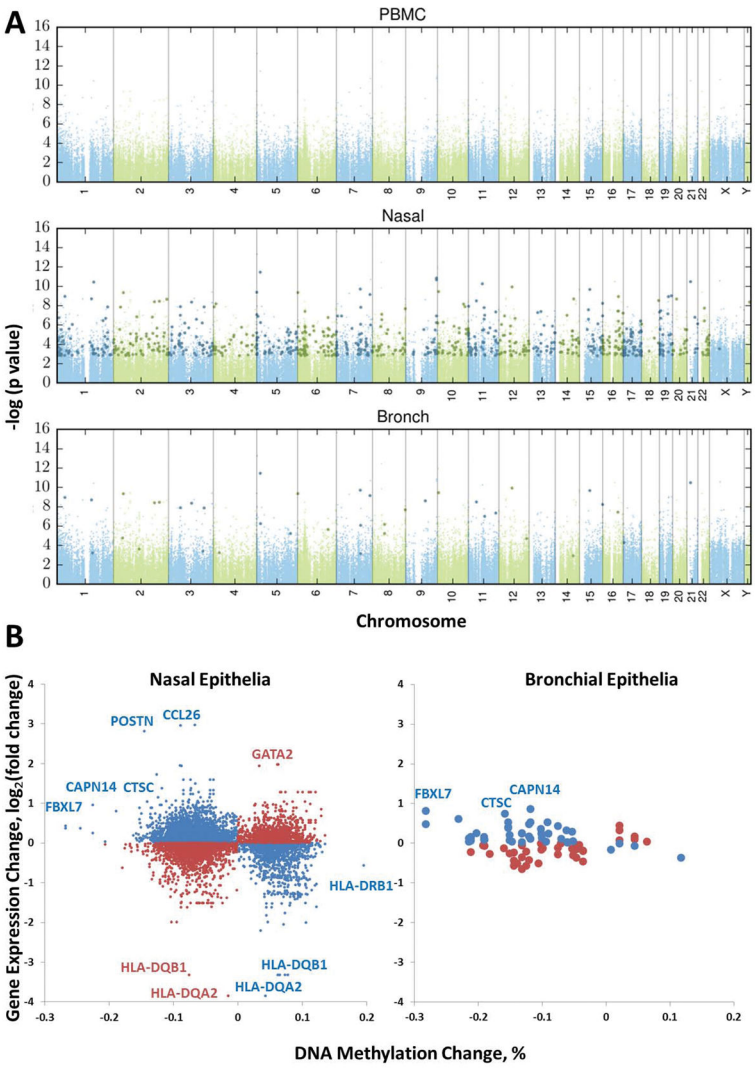

Figure 1.

Asthma-associated DNA methylation and gene expression changes in nasal epithelia of non-Hispanic White nonsmoker allergic asthmatics. **(A)** Differentially methylated single-CpG probes (DMPs) in bronchial and nasal epithelia, but not PBMCs, are associated with asthma after controlling for age, gender, technical variables, and batch effects in Caucasian adult subjects. Manhattan plot of the false discovery rate (FDR) adjusted p-values (q-values) for disease status (asthma/control) from the tissue-specific linear model. Probes with $q<0.05$ in the tissue-specific linear model are highlighted by darker larger symbols. **(B)** DNA methylation changes are associated with changes in gene expression in nasal and bronchial epithelia. Expression changes of genes nearest DMPs from part A. X-axis methylation difference is represented by the mean % methylation difference in asthma subjects compared to controls; y-axis expression difference is represented by the mean fold change in asthma subjects compared to controls (on the $\log_2$ scale). The blue symbols represent hypomethylated genes that were associated with increased gene expression as well as some hypermethylated genes associated with decreased gene expression. The red symbols

represent methylation changes that were not associated with expected gene expression differences. Reprinted with permission of the American Thoracic Society from Yang et al.[121] Copyright © 2017 American Thoracic Society. The American Journal of Respiratory and Critical Care Medicine is an official journal of the American Thoracic Society.

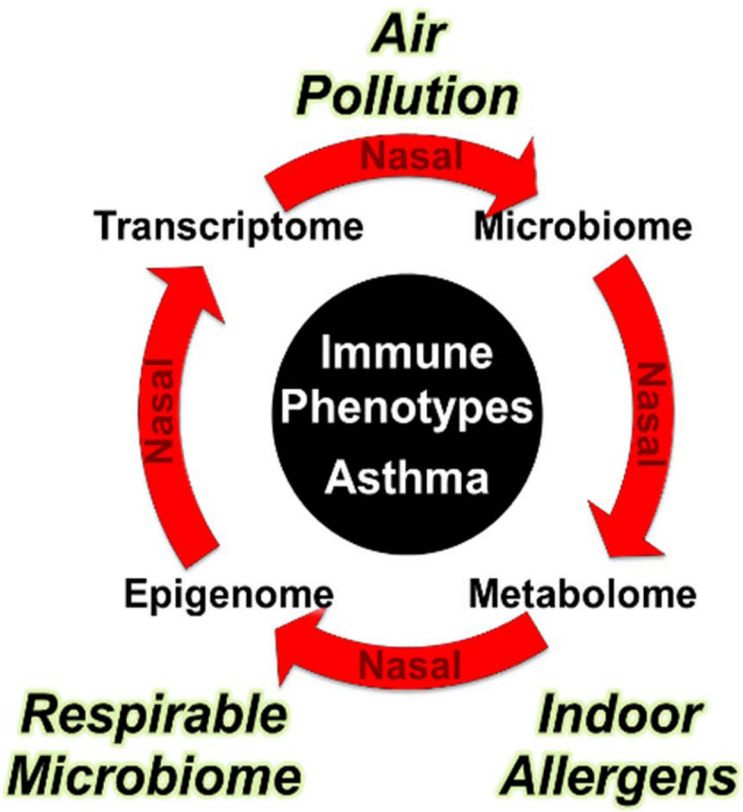

Figure 2.

Conceptual approach to integrative analysis of exposures and the epigenome/transcriptome in asthma. Sophisticated network approaches will be required to assess how environmental exposures (air pollution, allergens, microbiome), in the context of genetic susceptibility, interact to lead to changes in the epigenome and transcriptome, and ultimately to Th_2 cell skewing and specific disease phenotypes in asthma. Our ability to predict, prevent, and control asthma will be substantially advanced by understanding the complex interactive relationships of the microbiome, allergens, and air pollutants with the dynamic biological responses in the airways that predispose toward a Th_2 phenotype.