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Environmental Epigenetic of Asthma – An update

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Abstract

Asthma, a chronic inflammatory disorder of the airway, is influenced by interplay between genetic and environmental factors now known to be mediated by epigenetics. Aberrant DNA methylation, altered histone modifications, specific microRNA expression, and other chromatin alterations orchestrate a complex early-life reprogramming of immune T cell response, dendritic cell function, macrophage activation, and a breach of airway epithelial barrier that dictates asthma risk and severity in later life. Adult-onset asthma is under analogous regulation. The sharp increase in asthma prevalence over the past two or three decades and the large variations among populations of similar racial/ethnic background but different environmental exposures favors a strong contribution of environmental factors. This review addresses the fundamental question of whether environmental influences on asthma risk, severity, and steroid resistance are partly due to differential epigenetic modulations. Current knowledge on epigenetic effects of tobacco smoke, microbial allergens, oxidants, airborne particulate matter, diesel exhaust particles, dietary methyl donors and other nutritional factors, and dust mites is discussed. Exciting findings have been generated by rapid technological advances and well-designed experimental and population studies. The discovery and validation of epigenetic biomarkers linked to exposure and/or asthma may lead to better epigenotyping of risk, prognosis, treatment prediction, and development of novel therapies.

Keywords

pulmonary disorder; traffic-related pollutants; polycyclic aromatic hydrocarbons; microbial and viral infection; lipopolysaccharide; endotoxin; oxidants early-life programming; nutrition; maternal exposure; T helper cells; dendritic cells; macrophages; lung epithelial cells; phenotype plasticity; developmental basis of disease; gene-environment interaction; DNA methylation; histone modification; miRNA; chromatin remodeling; allergen; inflammatory response

Asthma is still poorly understood. It is not one disease but many, with some known but many unidentifiable causes underlying its development and manifestation. As such it is referred to as a complex disease for which an individual's risk is believed to be determined by a complicated interplay of one's genetics and environment exposures. The genetic ¹ or environmental ² explanations of asthma have been discussed and debated for many years.

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Declaration of interest

The authors have nothing to disclose.

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Our recent understanding of epigenetics as a mechanism mediating gene-by-environment interaction offers new opportunities to advance novel concepts and re-examine established ones about this disease^{3,4}. In this review, we will first discuss some key features of asthma, the basic principles of epigenetic regulation, and theories of phenotype/developmental plasticity before summarizing recent advances in environmental epigenetics that influence asthma pathogenesis. We will address future challenges and opportunities for the field, focusing on those that may help prevent asthma.

Asthma: main features and disease impact

Asthma, a chronic inflammatory disorder of the airway, is characterized by recurring episodes of airflow obstruction, wheezing, coughing, and shortness of breath⁵. However, its symptoms are highly variable and the etiologies of asthma and their interactions remain largely uncertain⁶. Asthma can cause intermittent episodes or follow a more chronic course, can occur with or without atopy, usually has its onset in childhood but sometimes is not recognized until adulthood, and can be corticosteroid-sensitive or -resistant. The heterogeneity of asthma suggests it is influenced by a multitude of factors, including genetics, family history, age, gender, socioeconomic status, race and/or ethnicity, and a host of recognized environmental factors.

The prevalence of childhood and adult-onset asthma has increased dramatically during the last two to three decades in both developed and developing countries⁷, although there are signs of a possible leveling off of its prevalence⁸. World-wide prevalence estimates are between 100 and 150 million people⁹. This disorder is clearly more prevalent in more developed countries such as the United States of America¹⁰.

Asthma has become a major health and economic burden of our nation, disproportionately affecting minorities in inner city communities, creating concerns about major health disparities¹¹.

Immune cell dysfunction and airway hypersensitization

Although the etiology of asthma is multifactorial, the role of specific T cells and their cytokines in the pathogenesis of allergic asthma is now well recognized¹². The infiltration and accumulation of polarized CD4⁺ T helper 2 (Th2) cells, degranulated mast cells, and eosinophils in the bronchial mucosa are the pathological features of allergic asthma. Allergic asthma starts with an influx of naïve CD4⁺ T cells and eosinophils into the bronchial mucosa. The priming of the naïve CD4⁺ T cells to differentiate into pro-inflammatory T helper 2 type (Th2) cells instead of the infection-fighting Th1 cells in the T cell repertoire by allergen-activated dendritic cells is an important proposed mechanism¹³. The progressive increase in the commitment of CD4⁺ T cells towards a Th2 phenotype is accompanied by an upregulation of the Th2 inflammatory cytokines such as interleukin (IL)-4, IL-5, IL-9, and IL-13 and an increased expression of the transcriptional factor GATA3¹². In parallel, the Th2 cells shut off the expression of interferon- (IFN) and other Th1 cytokines, such as IL-2. The recent discovery of Th17 in the mediation of corticosteroid-resistant asthma sheds new light on neutrophilic asthma¹⁴. In short, a skewed programming of CD4⁺ T cells towards a Th2 or aTh17 phenotype is a primary cause of asthma and other immuno-dysfunction of the airway.

As a counterbalance, naïve CD4⁺ T cells can differentiate into Foxp3⁺ T regulatory cells (Tregs) upon TGF- stimulation. This cell type confers immune tolerance, prevents autoimmunity, and dampens allergic responses. It suppresses a Th2 response but can promote a Th17 response. Thus, induction of Tregs differentiation can ameliorate asthma via

the suppression of a Th2 response, but this strategy may be limited by the potential activation of a Th17 response ¹⁵.

In addition to the T cell dysfunction, the interaction between epithelial cells and dendritic cells (DCs) in the airways plays a crucial role in determining the ability of inhaled allergens to initiate and maintain allergic Th2 cell-mediated responses. Upon challenge with an allergen, airway epithelial cells release chemokines and cytokines to attract and activate the DCs, which migrate and settle in the basolateral space of the airway epithelium. The DCs send processes into the airway lumen and sample for allergens. Activated DCs then migrate to regional lymph nodes to interact with regulatory cells and ultimately to stimulate Th2 cell production by naïve T cells. The DCs function as key antigen-presenting cells that translate the signal from allergens on the airway surface to T cells ^{16,17}.

Last, an often forgotten cell type involved in asthma is alveolar macrophages. They are the predominant immune effector cells residing in the airways. They play the dual role of activating inflammatory responses sufficient to eliminate the pathogens/allergens and suppressing the responses to allow for tissue repair and remodeling after inflammatory insults to the airway ^{18,19}. In the asthma exacerbation role, they can be activated by allergens to release inflammatory mediators and cytokines that amplify the inflammatory response ¹⁹. In the suppressive role they can ingest apoptotic inflammatory or structural cells to reduce inflammation or release cytokines and nitric oxide to promote Th1 development ¹⁹.

Genetics and asthma genes

Asthma risk is influenced by genetics. Having a parent with asthma doubles a child's risk of asthma and having two affected parents increases the risk fourfold ²⁰. The greater concordance of asthma among monozygotic twins as compared with dizygotic twins further supports this genetic influence ²¹. Findings from multiple studies of the genetic association of asthma identified 43 replicated asthma genes ²². The most frequently replicated of these genes are *TNF*, *IL4*, *FCERB*, *ADAM 33*, and *GSTP1*. Other genes identified are *DPPI10*, *GPR154*, and *PHF11* by linkage and fine mapping ²² and *ORMD3*, *IL1RL1*, and *PDE4D* by genome-wide association studies ²³. Most of these genes are associated with inflammation or a shift of the immune system toward a Th2 response, while others are surrogate biomarkers of inflammation. None alone is sufficient to predict or explain asthma, and there is a high degree of heterogeneity in the association of these genotypes among affected individuals or populations ²². These findings suggest that asthma genes interact in a complex manner to regulate the risk and severity of the disorder and that genetics alone is insufficient to fully explain inter-individual or inter-population variations of the disease ¹. The missing explanation could reside in gene-by-environment interactions ²⁴, which are now believed to be mediated by epigenetic mechanisms ^{3-5,25}.

Key environmental factors associated with asthma

The rapid increase in the prevalence of asthma throughout the world- over only the past few decades ⁷, the huge variations observed among populations with a similar racial/ethnic background but different environmental exposures ²⁶, and the marked increase in the frequency of occupational asthma ² all point to the dominance of environmental factors in asthma etiology. Additionally, several emerging hypotheses, such as the early-life origin of asthma ^{4,27}, the hygiene hypothesis ^{28,29}, and the artificial habitat hypothesis ³⁰, all require explanations involving environmental contributions to asthma etiology.

Living in a developed country is a strong risk factor for asthma ¹⁰. This increased risk may, in part, be related to potent indoor and outdoor allergens and irritants present in such an

environment. Outdoor allergens and air pollutants that have been shown to trigger or exacerbate asthma include microbial and viral pathogens, airborne particulates, ozone, diesel exhaust particles (DEP), pollens, outdoor molds (such as *Alternaria alternata*), environmental tobacco smoke (ETS), cold air, and humidity ^{2,31}. Equally important are a host of indoor allergens that have been demonstrated to induce airway inflammation, such as those derived from dust mites, cockroaches, mice, and pets; particles generated from indoor combustion of tobacco, wood, and other plant fuels; and biological agents such as indoor endotoxin, products from gram-positive bacteria, and 1,3- β -glucans from molds ². Other environmental factors impacting asthma include pharmaceuticals (e.g., paracetamol ³²) and a variety of nutrients and dietary agents (e.g., omega-6 polyunsaturated fatty acids, saturated fat, vitamins C and D, β -carotene, magnesium, selenium, sodium, and components in a Mediterranean diet ³³). Worthy to note is that many of these indoor and outdoor asthma inducers/triggers also have demonstrable reprogramming effects on the immature airway during early life, leading to altered asthma risk in later life (see next section).

Moreover, occupational asthma that accounts for 5% to 15% of cases of asthma in adult workers has more than 250 suspected causative agents ², including isocyanates, flour, grain dust, airborne particles, colophony, latex, animal dander, aldehydes, and wood dust ^{2,34,35}. The severity of such occupational asthma is usually dependent on the concentration of the allergen and the duration of exposure. However, because many workers tend to change their jobs once they develop the disease, occupational asthma is under-diagnosed in the general population. Unfortunately, for many, the symptoms can persist for years after the exposure is removed, thus significantly affecting the health and socioeconomics of our work force.

Early-life origin of asthma -Windows of programming

Most cases of asthma are now considered to originate in early-life and therefore belong to a long list of complex diseases that are “programmable” by specific early-life environmental exposures ³⁶. The prenatal period (during growth of airways and development of the immune system) is a critical window of programming. In this regard, maternal exposure to ETS, traffic related-pollutants, viral infection, dust mites, and certain nutritional factors during pregnancy have been shown to increase the risk of asthma in offspring ^{33,37–40}. The second critical window is during early childhood, especially during the first year of life (during the expansion of alveoli and rebalancing of the immune responses). Thus, severe lower respiratory tract viral infections, exposure to airborne environmental irritants (ETS, DEP), dust mite allergens, and therapeutics (paracetamol or acetaminophen), and deficiency in some nutritional elements such as vitamin D ^{33,38,40–45} during infancy or early childhood have been shown to elevate childhood asthma risk. In contrast, exposure to dog or cat allergens is associated with protection from later childhood wheeze in some ⁴⁶, but not all ⁴⁷, cohort studies. Additionally, exclusive breastfeeding for longer than four months ^{48,49} and intake of probiotics that promote beneficial intestinal microbiota ^{50,51} have modest protective effects against wheeze and asthma. Synergistic effects among allergens/irritants have been observed. For example, exposure during infancy to indoor combustion-related pollutants has been reported to sensitize children to dust mite-induced asthma in later childhood ⁵². This type of interaction is worthy of further investigation since most exposures comprise a mixture of allergens or inducers.

Similarly, adult-onset asthma is under early-life influences ^{53–55}. Respiratory infections during infancy are associated with a greater incidence of chronic obstructive lung disease ^{56,57}. Prenatal active or passive exposure to tobacco smoke ⁵⁸ and traffic-related exposure to polycyclic aromatic hydrocarbons (PAH) ⁵⁹ are associated with low birth weight and very preterm birth, two conditions that have positive correlations with adult lung deficiencies ^{56,60}.

Thus, it has become clear that most cases of asthma, of both childhood and adult onset, originate in early life. What remains elusive is how exposure in early life can permanently change one's susceptibility to asthma throughout life. One proposed mechanism is Barker's hypothesis of developmental plasticity^{36,61}, which contends that during early life, in response to an environmental disruption (e.g., infection, hyponutrition, ETS), most bodily organs, through the use of developmental plasticity, can establish an altered phenotype that is expected to better suit the needs of later life. Such responses are longer-term adjustments made by an organ that is based on present guesses about probable future needs. These adjusted phenotypes are usually beneficial to the health of the individual. However, exceptions arise when early guesses do not match later-life demands. A high degree of mismatch between the "adaptive trait" established in early life and demands in later life may increase the risk of developing disease. In the case of asthma, it has been proposed that exposures to pathogens, metabolic changes, and other environmental factors during pre- or postnatal life trigger the early airways to undergo a different course of development resulting in a phenotype of increased sensitivity to allergens or irritants, hyper-responsiveness, and a skewed Th2 response⁴. These alterations in airway and Th cell phenotype create a lasting vulnerability for developing asthma in later life.

The mechanisms underlying environmental reprogramming of the early airway and T cell phenotype remain unclear. However, a growing body of literature now suggests that the link resides in epigenetics, which is responsible for partitioning and remodeling of the genome into active and inactive domains and creating long-lasting changes in transcriptional programs of the airway and Th cells that favors asthma pathogenesis⁶²⁻⁶⁷.

Mechanisms of epigenetic reprogramming

Epigenetics is the study of mitotically heritable changes in phenotype (alterations in gene expression) that occur without direct alterations of the DNA sequence^{68,69}. These epigenetic changes include methylation of DNA by the covalent addition of a methyl group to a cytosine residue in a CpG site⁷⁰; the post-translational modification of the amino acid tails of histones by acetylation, phosphorylation, methylation, sumoylation, or ubiquitylation⁷¹; and aberrant expression of microRNAs (miRNAs), each of which is capable of post-transcriptionally regulating the expression of a cohort of cognate target genes⁷². Collectively, these three major epigenetic mechanisms affect interactions of DNA with transcriptional factors, transcript stability, DNA folding, nucleosome positioning, chromatin compaction, and higher-order nuclear organization in a manner that determines whether a gene or a set of genes is silenced or activated and when and where a gene will be expressed. They therefore play crucial roles in determining the transcriptional programs of differentiating or differentiated tissues. Before we discuss examples of early-life reprogramming of the airways and its related immune responses by environmental agents via epigenetic mechanisms^{4,5,25,73}, we will brief outline how these mechanisms can alter gene expression and hence the phenotype of cells and organs on a long-term basis.

CpG dinucleotides are underrepresented in the mammalian genome (1–2%) but tend to cluster as CpG islands in gene promoter regions. Hypermethylation of promoter CpG islands is commonly associated with transcriptional silencing, possibly because the methylated promoter has reduced affinity for transcriptional factors⁷⁴ and increased affinity for methylated DNA-binding proteins (e.g., MeCP2, MBD1, MBD2, MBD3, and MBD4), which further recruit histone deacetyltransferases and other corepressors. Methylated promoters also are associated with unique repressive histone markers⁷⁵, which classically include trimethylation of histone 3 (H3), lysine (K) 9, and H3-K27. Conversely, unmethylated promoters are associated with gene activation, reduced affinity for methylated DNA-binding proteins, and histone marks associated with active chromatin, including

acetylated H3-K9 and trimethylated H3-K4. Histone modifications are believed to mediate more rapid responses to environmental influences ^{4,76,77}, whereas DNA methylation mediates gene silencing over a longer time frame. Thus, the two mechanisms work closely in gatekeeping the active and inactive states of a gene or parts of the genome.

DNA methylation requires the activity of DNA methyltransferases (DNMTs). DNMT1 facilitates the replication of the DNA methylation pattern between cell generations (maintenance methylation), and DNMT3a and DNMT3b mediate *de novo* methylation of DNA ^{78,79}. The mechanism of DNA demethylation is less clear. Loss of binding to methylated DNA-binding proteins may allow the promoter to enter into a transcriptional state. However, the association of methylated DNA with MBD2 or MBD4 has been proposed to induce active DNA demethylation, a hypothesis currently under active debate ^{80,81}.

Histone modifications (marks) are believed to change gene expression by remodeling the chromatin of the promoter and/or the coding region of target genes. They serve to recruit specific chromatin modeling enzymes (DNMTs, demethylases), methylated DNA binding proteins, and shift the position of the nucleosomes ^{82,83}, thus maintaining either an active or an inactive transcriptional environment. They are known to transduce extracellular signals (such as IGF1 ⁸⁴) to activate genomic events. Histone modifications work conjointly with DNA methylation to achieve short- and long-term changes in transcriptional programs through transient or permanent reorganization of the chromatin architecture ⁸⁵. Histones are modified by specific enzymes that include histone acetyltransferases (HAT), deacetylases (HDAC), and methyltransferases (HMT) ⁸⁶. Their antagonists hold great promise as epigenetic pharmaceuticals.

MicroRNAs (miRNAs) function as post-transcriptional regulators of cognate target gene expression ⁷². They are a class of small non-coding RNAs produced from either their own genes or introns/exons of other genes. They bind to target mRNAs with complete or incomplete complementarities and/or degrade/modify target mRNAs and modulate protein translation ⁸⁷. It is now known that one miRNA may target hundreds of mRNAs and that one mRNA may be regulated by different miRNAs. Thus, although the field is still in an early stage of development, it has great potential to reveal a new level of epigenetic regulation.

Epigenetics regulates the immune responses associated with asthma

Epigenetics is now recognized as a key mechanism underlying the establishment and maintenance of the Th2 bias in asthma patients ^{5,88}. Exposure to allergens induces an immune response that triggers the differentiation of a naïve Th cell into a Th2 cell, expressing the cytokines IL-4, IL-5, and IL-13 responsible for the allergic response ⁸⁹. Loss of DNA methylation and increased association with activating histone marks conjointly establish and maintain a euchromatin configuration at the Th2 locus of Th2 cells, allowing recruitment of the transcriptional machinery to this region for a rapid and coordinated expression of the Th2 cytokines. The early response is marked by rapid increases in *IL-4* expression, as the GATA3 transcriptional factor binding sites within the first intron of the gene loses CpG methylation and the *IL-4* locus gains H3-K9 acetylation and trimethylation of H3-K4 ⁹⁰⁻⁹³. With lineage commitment, additional demethylation occurs in the 5' end of the gene, which is essential for sustaining a high level of IL-4 expression ⁹¹. In parallel, the expression of IFN- γ in Th2 cells is silenced by repressive histone marks ⁹² and increased promoter CpG methylation ^{94,95}. In contrast, a Th1 differentiation is associated with methylation of the 3' end of the *IL-4* locus ⁹¹. Furthermore, Th2 polarization is associated with loss of IFN- γ expression, which is thought to be mediated by methylation of specific

CpGs in its promoter region^{94,95}. Specifically, methylation of CpG⁻⁵³, an AP-1-binding site in the proximal promoter of *IFN*, results in inhibition of CREB and ATF2/c-Jun binding to this *cis*-regulatory element and sustained gene silencing^{95,96}. Hence, mounting evidence suggests that the development of a polarized Th2 phenotype is a result of major chromatin remodeling brought about by multiple, coregulatory epigenetic changes on genes regulating Th differentiation.

Moreover, the Th1/Th2 ratio is exquisitely sensitive to histone acetylation/deacetylation regulation⁷³. In this regard, inhibition of endogenous HDAC activity with trichostatin A (TSA) can shift recall responses toward a more Th2-like phenotype by changing the Th1/Th2 ratios three- to eight-fold and increasing Th2-associated (IL-13, 139%; IL-5, 168%) and reducing Th1-associated recall responses (IFN- γ , 76%; CXCL10, 47%)⁹⁷. Of significance to glucocorticoid-resistant asthma, upregulation of class II HDAC restores steroid responsiveness in the airways⁹⁸. Treatment with inhibitors for both class I and II HDACs, but not those only effective for class I enzymes, induces FoxP3⁺ production and boosts the suppressive function of Foxp3⁺ regulatory T cells (Tregs) on Th2-mediated allergic response⁹⁹. In addition to Th2 polarization, a recent study has shown that human Tregs can differentiate into Th17 cells via epigenetic plasticity that can be modulated by histone/protein deacetylase activity¹⁰⁰. It has been noted that neurophilic asthma may involve Th17 polarization¹⁰¹. Taken together, these findings have significant clinical ramifications, as new anti-asthma strategies seeking to target specific HATs/HDACs may have great utility in the future management of asthma¹⁰².

Last, emerging evidence suggests that miRNAs are involved in the pathogenesis of immunologic diseases, including asthma¹⁰³. A single nucleotide polymorphism at the 3' untranslated region of the human leukocyte antigen-G (HLA-G), an asthma-susceptibility gene¹⁰⁴, was shown to be a putative target site for three miRNAs: miR-148a, miR-148b, and miR-152¹⁰⁵. A recent study demonstrated that the inflammatory airway of a lung-specific IL-13 transgenic mouse overexpressed miR-21 and underexpressed miR-1¹⁰⁶. It also revealed that *IL-12p35*, a predicted target of miR-21 and a cytokine germane to Th cell polarization, was significantly downregulated in the mouse inflamed airway. In human bronchial epithelial cells (HBECs), miR-146a expression was found to be upregulated in response to TGF- β 1 plus cytomix (a mixture of IL-1 γ , IFN- γ , and TNF- α)-induced apoptosis and that a mimic for this miR can upregulate Bcl-XL and STAT3 phosphorylation, improve HBEC survival, and contribute to tissue repair and remodeling¹⁰⁷. Furthermore, selective knockdown of miR-126 expression was shown to suppress the asthmatic phenotype, resulting in diminished Th2 response, inflammation, airways hyperresponsiveness, eosinophil recruitment, and mucus hypersecretion¹⁰⁸. At the molecular level, downregulation of miR-126 inhibited Th2 polarization by increasing the expression of POU domain class 2–associating factor 1, which activates the transcription factor PU.1, leading to loss of GATA3 expression. These new findings support the notion that miRNA-based oligonucleotide therapies will be an emerging class of anti-asthma regimens.

In aggregate, multiple epigenetic mechanisms regulate a handful of asthma-related genes known to initiate and maintain the asthma phenotype and its symptoms. Table 1 summarizes these genes and their relevance to the disorder.

Environmental factors exert epigenetic influences on asthma

Recent findings of the regulation of multiple aspects of asthma pathogenesis by epigenetics raise the fundamental question about whether environmental influences on asthma risk or its manifestation are mediated through similar epigenetic changes found to contribute to this disorder. Current knowledge of the effects of environmental agents found to be

epigenetically active and to contribute to the pathogenesis of asthma is summarized below and in Table 2.

1. Tobacco smoke

Exposure to tobacco smoke represents a major risk factor for the development of asthma^{109,110}. Enhanced sensitization to allergens has been observed in humans and laboratory animals exposed to tobacco smoke. Early-life exposures clearly elevate asthma risk in later life¹¹¹. The epigenetic action of tobacco smoke can be direct or indirect via the induction of oxidative stress.

One epigenetic action of tobacco smoke is mediated through the disruption of HAT/HDAC homeostasis in immune cells of the airways. A recent study comparing biopsies and bronchoalveolar lavage (BAL) alveolar macrophages from normal nonsmoking subjects and age-matched healthy tobacco smokers found that tobacco smoke suppressed HDAC2 expression and overall HDAC activity and enhanced expression of inflammatory mediators such as GM-CSF, IL-8, and IL-1 β -induced TNF- α ¹¹². Importantly, tobacco smoke markedly attenuated dexamethasone inhibition of cytokine release in these cells and hence may cause steroid resistance. Treatment of the macrophages with the HDAC inhibitor TSA reversed the pro-inflammatory changes and glucocorticoid responsiveness in the macrophages, supporting the possible usefulness of this class of drug as an adjuvant for asthma treatment. Since the treatment of a macrophage cell line with hydrogen peroxide mimicked the effects of tobacco smoke on HDAC activity and glucocorticoid responsiveness, it has been suggested that part of the action of tobacco smoke can be mediated via the induction of oxidative stress. Since macrophages function to fine tune allergen-induced airway inflammation (see above), an epigenetic disruption of their function likely contributes to asthma and other airway diseases.

In addition to modulating HAT/HDAC activities, tobacco smoke can exert epigenetic action via alteration of DNA methylation status in gene promoters or regulatory sequences. Multiple studies have shown that tobacco smoke induces promoter hypermethylation of p16 [INK4a], a purported tumor suppressor involved in cell-cycle regulation in non-small cell lung cancer cells^{113–115}. Other lung cancer-related genes whose methylation status can be affected by smoking include *CYP1A1*¹¹⁶, *RASSF1A*¹¹⁷, and *FHIT*¹¹⁸. However, it remains to be determined whether these epigenetic changes are the result of exposure to tobacco smoke or are pathological changes associated with carcinogenesis.

A more direct piece of evidence linking tobacco smoke and DNA methylation can be found in a recent study that reported hypomethylation of the *monoamine oxidase (type B)* (*MAOB*) promoter in peripheral blood mononuclear cells of smokers (former and current) as compared with non-smokers¹¹⁹. Moreover, the degree of methylation of the *MAOB* promoter was inversely correlated with platelet expression of MAO-B protein. Of significance to our understanding of the long-term impact of epigenetic changes, hypomethylation of the *MAOB* promoter persisted long after (>10 years) the individuals in this study had stopped smoking.

DNA methylation may also be an epigenetic mechanism that can explain the life-long effect of exposure to tobacco smoke *in utero* on asthma risk^{120,121}. Gilliland and associates¹²² recently examined DNA methylation status in buccal cells from a cohort of children born to mothers who smoked or did not smoke during pregnancy. Children exposed to maternal smoking had lower methylation of the *AluYb8* repeat element, indicating global DNA hypomethylation. In addition, they also identified, using a CpG loci screen, differential methylation of eight genes between those children exposed and those not exposed *in utero* and validated the hypermethylation of two genes, *AXL* and *PTPRO*, in the exposed children.

AXL is a receptor tyrosine kinase that promotes antiapoptosis, mitogenesis, invasion, and cell survival¹²³, whereas PTPRO is a protein tyrosine phosphatase receptor involved in differentiation and axonogenesis of central and peripheral nervous system neurons during gestation¹²⁴. At this point, it is unclear how these genes function to alter asthma risk. However, of special interest to the concept of gene-by-environment interaction, differences in smoking-related effects on *LINE1* methylation were observed only in children with the common *GSTM1* null genotype, thus suggesting that variations in genotype involved in the metabolism of tobacco smoke can interact with epigenetics to alter asthma risks in children born to mothers who smoked during pregnancy.

2. Polycyclic aromatic hydrocarbons (PAH)

PAH are one of the most widespread classes of pollutants of the environment and in food¹²⁵. They are present in crude oil, coal, and tar deposits and are derived from incomplete combustion of fossil fuel, oil, garbage, and cigarettes. They are major components of airborne particulate matter (PM) of urban aerosols and are widely present in food products, including grains, vegetables, oils, and fats. PAH are emitted into the air during the production of coke and aluminum. Cooked meats are contaminated when they are charcoal-grilled, roasted, or smoked. Among the PAH, benzopyrene (BaP) is often used as a prototype PAH for many experimental studies.

The association of asthma with particulate air pollutants, DEP, the World Trade Center disaster, maternal smoking, and exposure to ETS, coke manufacturing, and firefighting is well documented^{126–132} and may well be related to the PAH component of these environmental toxicants/pollutants. However, the evidence that indicates PAH is a major contributing factor of asthma is just emerging. This scarcity of information is due in part to the lack of mechanistic studies and accurate biomarkers of exposure.

Using a restriction enzyme–based microarray approach, Sadikovic and Rodenhiser reported that BaP-induced hypomethylation of a number of genomic repeats and sequence-specific hypo- and hypermethylation changes in four breast cancer cell lines¹³³. The investigators were able to correlate some of these changes to cell growth and the p53 status of the cell lines. Unfortunately, they subsequently discovered that this array approach was compromised by the ability of BaP to form adducts at CpG dinucleotides, thus inhibiting restriction-enzyme activities and PCR amplification¹³⁴. They then turned to investigating the impact of BaP on H3K9 acetylation at a genome-wide level in the MCF-7 breast cancer cell line and found that BaP induces hypo- and hyperacetylation in genes belonging to networks regulating gene expression, DNA replication and repair, and carcinogenesis¹³⁵. Within these networks are genes involved in the organization and remodeling of chromatin, including *MTA3*, *HDAC1*, *ATRX*, *MBD2*, and *MBD3*. These findings are in agreement with previous studies reporting that BaP can decrease global DNA methylation¹³⁶, inhibit DNA methyltransferases *in vitro*¹³⁷, and interfere with recruitment of the methylation machinery^{138,139}. Although these studies have firmly established an epigenetic effect for BaP, their direct relevance to asthma remains debatable.

In a recent study¹⁴⁰, we identified, using an unbiased screening method, a novel epigenetic marker for PAH-associated asthma. Hypermethylation of the *acyl-CoA synthetase long-chain family member 3* (*ACSL3*) promoter in umbilical cord white blood cells of children born to mothers with variable, but well-documented, levels of PAH exposure was highly correlated with increased maternal exposure and risk of developing asthma symptoms before age 5. *ACSL3* belongs to the acyl-CoA synthetase long-chain (ACSL) family of genes that encode key enzymes in fatty acid metabolism¹⁴¹. It is expressed in lung and thymus tissue^{142,143}. Thus, hypermethylation of this gene in T helper cells or lung tissues is expected to diminish fatty acid utilization and beta-oxidation-energy production and

possibly influence the phospholipid composition of membranes. Interestingly, *ACSL3* is located in 2q36.1, which has recently been shown to be associated with regions of the asthma-susceptibility loci in specific populations^{144,145}.

Lastl, two CpG-rich regions in the promoter of *INF-* were found to undergo hypermethylation when human airway smooth muscle cells or lung cancer cells were exposed to BaP. *INF-* promoter in umbilical cord white blood cell DNA was found to associate positively with maternal PAH exposure and increased risk of childhood asthma (Ho, unpublished data). Since silencing of *INF-* is directly linked to the development of Th2 polarization, these findings should provide a new angle to the investigation of environmental genetics of asthma.

3. Microbial infection, inflammation, and oxidative stress

Both epidemiological and experimental studies have shown that microbial exposure in early life can protect against asthma but that exposure in later life predisposes to the disorder^{146–149}. These contradicting outcomes could be explained by multiple mechanisms, including developmental plasticity altered during early life by epigenetic events. The first of such mechanisms may be related to the well-documented fact that infections promote the generation of oxidants^{150,151} and proinflammatory mediators¹⁵² in the airways. These intermediates in turn can exert epigenetic modifications on transcriptional programs of cytokines. In this regard, damages by oxidants are known to trigger methylation. The formation of hydroxymethylcytosine as a result of oxidative stress or the generation of halogenated cytosines as a result of the release of hypochlorous acid from neutrophils or of hypobromous acid from eosinophils can lead to methylation¹⁵³. Thus, an increase in oxidant could promote cytosine methylation-mediated gene silencing that may have long-lasting effects.

Oxidants and proinflammatory mediators also regulate histone acetylation/deacetylation balance in the airways^{154,155}. H_2O_2 can alter the histone acetylation and deacetylation balance via post-translational modification of HDACs. An imbalance in HAT/HDAC stoichiometry contributes to the enhancement of IL-1 β -stimulated inflammatory cytokine production (IL-8, IL-6, CXCL1, CXCL2, and CXCL3) in the inflamed airways. Modifications in the histone marks associated with gene loci of these cytokines can produce long-lasting epigenetic effects in their transcriptional programs.

A second explanation may be related to the biphasic nature of the response of the innate immune system to endotoxin released from bacterial cells. Prior exposure of innate immune cells like monocytes/macrophages to small amounts of endotoxin causes them to become refractory to subsequent challenges by endotoxin, a phenomenon known as “endotoxin tolerance.” This may explain why endotoxin exposure is associated with protection from developing asthma in some studies^{39,154,156} but with the development or exacerbation of asthma in others¹⁵⁷. An important mechanism underlying this endotoxin tolerance is epigenetic reprogramming of IL-1 β -mediated TNF release in these immune cells. Exposure to endotoxin or lipopolysaccharide (LPS) induces chromatin remodeling of the proinflammatory gene *IL-1* promoter nucleosome and epigenetic gene silencing of *TNF* that involves aberrant retention of the heterochromatin-binding protein 1, altered histone modifications, and loss of NF- κ B RelA/p65 binding to its promoter^{158–160}. A recent study further reported upregulation of miRNA-146a as a plausible mechanism of LPS priming¹⁶¹. Another study demonstrated that Akt1-regulated expression of let-7e and miR-155 may be responsible for tuning the LPS-driven *Toll-like receptor* signaling in macrophage sensitivity and tolerance to endotoxin¹⁶².

In summary, the relationship between microbial exposure and asthma is complex; the intricate interplays among infection, inflammation, oxidative stress and endotoxin tolerance likely involve multiple levels of epigenetic regulation.

4. Particulate matter, diesel exhaust particles, and other outdoor pollutants

Epidemiological studies have shown that PM, DEP, and other outdoor airborne pollutants are associated with adverse respiratory health effects, including asthma^{2,163,164}. Several of these have shown to exert their actions via epigenetics.

DEP is one of the major components of PM. In a mouse asthma model, a 3-week exposure to inhaled DEP was found to hypersensitize mice to intranasal exposure to *Aspergillus fumigatus*. The combinatory treatment increased IgE production and induced hypermethylation at CpG(−45), CpG(−53), and CpG(−205) sites of the *IFN-γ* promoter and hypomethylation at CpG(−408) of the *IL-4* promoter in DNA from splenic CD4⁺ cells¹⁶⁵.

DEP or PM can also exert their action in the airways via the induction of oxidative stress¹⁶⁶. Treatment of A549 cells (adenocarcinomic human alveolar basal epithelial cells) with either PM-10 or H₂O₂ increased IL-8 expression and release, which was augmented by co-treatment with TSA, a HDAC inhibitor, but blocked by co-treatment with antioxidant. Both PM-10 and H₂O₂ treatment increased HAT activity and the level of acetylated histone 4 and remodeled the *IL-8* promoter region. These data suggest that the action of PM-10 is mediated by oxidative stress, which in turn triggers histone acetylation-induced remodeling of the chromatin associated with cytokine release in the lungs¹⁶⁷.

Baccarelli et al.¹⁶⁸ found that increased exposure of elderly participants (718) to ambient particulate pollutants for a short duration (4h to 7d) was associated with DNA hypomethylation of *LINE-1*, but not *Alu*, repetitive elements in their blood DNA samples. Interestingly, black carbon, but not PM_{2.5}, showed this association. These findings lay the groundwork for future investigation of whether these global methylation changes or alterations in specific genes are linked to exposure-related health outcomes.

5. Diet and nutritional factors

In mammalian cells, during mitosis the maintenance of the fidelity of the methylation pattern in the newly synthesized DNA strand is dependent on the availability of diet-derived methyl donors and cofactors required for the synthesis of S-adenosylmethionine (SAM). The concentration of SAM affects DNA methyltransferase activities and prevents aberrant global hypomethylation of the genome, which could be a cause of congenital diseases and aging¹⁶⁹. In agouti mice, a deficiency in methyl donors or their coenzymes, such as choline, betaine, folic acid, and vitamin B12, *in utero* predisposed the offspring to many complex diseases^{170,171}. However, the evidence demonstrating that nutritional factors can directly influence epigenetic programming of T cells and airway tissues is still limited.

A recent report found that exposure of pregnant mice to a diet rich in methyl donors favored lymphocyte maturation into a Th2 phenotype and increased the risk of developing allergic airway disease in the offspring¹⁷². The maternal diet induced methylation changes in 82-gene loci in the offspring. Among these genes, *Runt-related transcription factor 3* (*Runx3*), a gene known to suppress allergic airway disease, was found to be hypermethylated, along with concordant transcriptional silencing of *Runx3* in progeny. These findings demonstrate that dietary factors can modify asthma risk through epigenetic mechanisms during a susceptible period of developmental reprogramming^{3,4}. They are in agreement with findings from a large-scale cohort study, the Norwegian Mother and Child Cohort Study (>32,000 children), which showed that maternal folic acid supplementation increased the risk of wheeze and lower respiratory tract infections in progeny up to 18 months of age¹⁷³.

In aggregate, these findings call into question of the safety of supplementing maternal diets with methyl donors or their coenzymes.

A growing body of evidence now suggests a protective effect of vitamin D against asthma^{174–176}, but little is known if its action is mediated via epigenetics. This line of investigation should prove promising in the future, since a combination of vitamin D with an epigenetic therapy may be highly effective.

6. Dust mites and other indoor allergens

An emerging concept for a mechanism potentially causing asthma is that the innate immune system inappropriately senses allergens as foreign and dangerous and responds with a programmed adaptive Th2 immune response. Toll-like receptors (TLRs) differentially sense microbial and viral bioproducts and act as sentinels for the activation of innate host defense pathways. Lipopolysaccharide (LPS), a cell-wall component of Gram-negative bacteria, activates cells through TLR4 and the common TLR adaptor protein myeloid-differentiation-primary-response-gene-88 (MyD88), resulting in activation of transcription and proinflammatory pathways. LPS is also a prominent constituent of asthma-inducing house dust mite (HDM) allergens and can instruct the immune response to inhaled antigen to generate Th2 responses.

Toll-like receptors (TLRs) act as sentinels for activating innate host defense in response to inhaled antigens and play a pivotal role in programming a Th2 immune response. Exposure to house dust mite antigens activates the TLR4 and increases the expression of a unique set of miRNAs that includes miRNA-16, -21, and -126¹⁰⁸. Selective blockade of miRNA-126 leads to amelioration of asthma symptoms and a diminished Th2 response, inflammation, and airway hyperresponsiveness through a miR-126-mediated suppression of GATA-3 expression. These data open doors for future asthma therapies based on micro-RNAs or their antagomirs.

The major indoor allergens include arthropod allergens, animal dander mammalian allergens (from pets or pests), and fungal allergens. Nevertheless, no information is available on their epigenetic action in the airways or asthma-related immune systems. Future research on how indoor allergens program airways and the immune system via epigenetics is of critical importance, as modern living involves spending nearly 90% of time indoors.

What are the gaps in data?

First, can we identify unique and specific epigenetic marks that are linked to each allergen or environmental inducers of asthma? Can these epigenetic changes be developed into exposure biomarkers or disease predictors? Can epigenetic biomarkers with high sensitivities and specificities for an environmental factor be used for formulating regulatory policies? How much overlap do environmental epigenetic biomarkers have among different classes of asthma inducers or triggers? Can environmental genetics contribute to our fundamental understanding of asthma etiology?

Second, when are the critical developmental periods of airway and immune cell programming by environmental factors for childhood and adult asthma? How long will the epigenetic memories last and are they reversible by later-life events, including removal of the environmental inducers, the use of epigenetic disruptors such as dietary methyl donors, and epigenetic therapeutics including HDAC inhibitors and miR antagomirs/antagomirs?

Third, once an environmental inducer is removed will its presumed long-lasting epigenetic action gradually disappear? Can this reversal be accelerated through the adoption of life-

style changes and/or treatment with targeted therapies? In this regard, the permanency of early-life programming and the effectiveness of later-life modifiers need to be understood.

Fourth, how can environmental epigenetics explain co-sensitization between two or more classes of allergens? Can it explain remission, tolerance, and treatment resistance? More important, can it be used to predict individual or population-based variability to susceptibility or treatment? In this regard, the identification of susceptible individuals or populations via epigenotyping will provide new measures for disease surveillance, prevention, and management. Furthermore, identification of the environmental culprit for an individual's asthma could lead to personalized management of the disease. If this can be extended to exposed populations such as schoolchildren, the elimination of the irritant or allergen in their environment will have significant public health ramifications.

Fifth, can epigenetic marks in surrogate tissues such as buccal cells, cord blood, amniocentesis fluid, and skin cells be used to predict the pathophysiological changes in the target tissues such as the airway and the immune cells? This question is critically important for advancing epidemiologic studies in large cohorts, especially those studying childhood asthma.

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Abbreviation

ATF2	Activating transcription factor 2
ACSL3	Acyl-CoA synthetase long-chain family member 3
ADAM33	ADAM metalloproteinase domain 33
AXL	AXL receptor tyrosine kinase
BCL-XL	BCL2-like 1
BaP	Benzopyrene
BAL	Bronchoalveolar lavage
CREB	cAMP responsive element binding protein 1
CXCL	Chemokine (C-X-C motif) ligand
INK4a	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)
CYP1A1	Cytochrome P450, family 1, subfamily A, polypeptide 1
DC	Dendritic cell
DEP	Diesel exhaust particles
DPP10	Dipeptidyl-peptidase 10
DNMT	DNA methyltransferase

ETS	Environmental tobacco smoke
FHIT	Fragile histidine triad gene
GATA3	GATA binding protein 3
GSTM1	Glutathione S-transferase mu 1
GSTP1	Glutathione S-transferase pi 1
H	Histone
HAT	Histone acetyltransferase
HDAC	Histone deacetylase
HMT	Histone methyltransferase
HDM	House dust mite
HBEC	Human bronchial epithelial cell
IGF-1	Insulin-like growth factor 1 (somatomedin C)
IFN-	Interferon-
IL	Interleukin
IL1RL1	Interleukin 1 receptor-like 1
c-Jun	Jun oncogene
LPS	Lipopolysaccharide
K	Lysine, FCERB
Membrane-spanning 4-domains	subfamily A, member 2 (Fc fragment of IgE, high affinity I, receptor for; beta polypeptide)
MECP2	Methyl CpG binding protein 2 (Rett syndrome)
MBD	Methyl-CpG binding domain protein
miRNA	microRNA
MAOB	Monoamine oxidase B
MYD88	Myeloid differentiation primary response gene (88)
GPR154	Neuropeptide S receptor 1
NF- b	Nuclear factor of kappa light polypeptide gene enhancer in B- cells 1
ORMD3	ORM1-like 3
PM	Particulate matter
PHF11	PHD finger protein 11
PDE4D	Phosphodiesterase 4D, cAMP-specific
PAH	Polycyclic aromatic hydrocarbon
PTPRO	Protein tyrosine phosphatase, receptor type, O
RASSF1A	Ras association (RalGDS/AF-6) domain family member 1
RUNX3	Runt-related transcription factor 3

SAM	S-adenosylmethionine
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)
Th	T helper
Treg	T regulatory
TLR	Toll-like receptor
TGF-	Transforming growth factor, beta
TNF-	Tumor necrosis factor, alpha

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Figure 1.

Environmental factor-induced T cell regulation of allergic airway responses. Inhaled allergens derived from environmental factors such as tobacco smoke, polycyclic aromatic hydrocarbons (PAH), endotoxin, diesel exhaust particles (DEP), particulate matter (PM), and dust mites in the immature or leaky airways are sampled by dendritic cells. The allergen-activated dendritic cells serve to prime the naïve CD4⁺ T cells to differentiate into pro-inflammatory T helper 2 type (Th2) cells instead of the infection-fighting Th1 cells in the T cell repertoire. The progressive increase in the commitment of CD4⁺ T cells towards a Th2 phenotype is driven by Th2 cytokines such as interleukin (IL)-4, IL-5, IL-9, and IL-13 and heightened expression of GATA3. In parallel, the Th2 cells shut off the expression of interferon- (IFN) and other Th1 cytokines such as IL-2. In neutrophilic, corticosteroid-resistance asthma, Th17 differentiation is increased. TGF- -driven naïve CD4⁺ T cells differentiating into Foxp3⁺ T regulatory cells confers immune tolerance and dampens allergic responses. Alveolar macrophages play a dual role in pathogen/allergen elimination and suppression of the responses for airway repair and remodeling. Allergen-triggered oxidative stress, dietary methyl donors, and nutritional factors such as vitamin D modulate these immune/airway reprogramming events. Cytokines and transcriptional factors colored red are known to be modulate by epigenetic events. ROR t, GATA-3, T-bet, and Foxp3 are transcriptional factor promoting the differentiation of the respective T cells. IL, interleukin; IFN- , interferon- ; Th cell, T helper cell; Treg cell, T regulatory cell; DC, dendritic cell; TGF- , transforming growth factor- .

Figure 2. DNA methylation and histone modification collaborate in regulating gene expression
 DNA methylation refers to the covalent addition of a methyl group to a cytosine (C) residue in a CpG dinucleotide (black circles = methylated C; open circles = unmethylated C). The carboxyl ends of histones have specific amino acids that are sensitive to post-translational modifications. These two major epigenetic mechanisms collaborate to package genes in euchromatin (active chromatin) or heterochromatin (silenced chromatin), a packaging that determines whether a gene or a set of genes is silenced or activated. CpG sites are underrepresented in the mammalian genome but tend to cluster as CpG islands (CGIs) in gene promoter regions. Hypermethylation of promoter CGIs is associated with transcriptional silencing (red X) because of loss of affinity for transcriptional factors (TF) and accessibility by the transcriptional machinery (represented by Pol II in this figure). The heterochromatin has increased affinity for methylated DNA-binding proteins (MBPs), which further recruit histone deacetyltransferases (HDACs), DNA methylases (DNMTs), and other corepressors. Methylated promoters are associated with unique repressive histone markers, which classically include trimethylation of histone 3 (H3), lysine (K) 9, and H3-K27. Unmethylated promoters are associated with gene activation (green arrow). They have reduced affinity for MBPs, increased affinity for histone acetylases (HATs), and histone marks associated with active chromatin, including acetylated H3-K9 and trimethylated H3-K4. Histone modifications are believed to mediate more rapid responses to environmental influences, whereas DNA methylation provides gene silencing over a longer time frame. Pol II, polymerase II; M, methylation; A, acetylation; H3, histone 3.

Table 1

Asthma-related genes known to be regulated by epigenetic mechanisms

Gene name	Mechanism of epigenetic regulation	Relevance to asthma	References
<i>IL-4</i>	Demethylation of an intronic sequence that binds GATA-3	Increases IL-4 secretion in Th lymphocytes	90
<i>IL-4</i>	An increase in H3-K9 acetylation and H3-K4 trimethylation	Increases lineage commitment of precursor Th cells to Th2 cells	92
<i>IL-4</i>	Extensive demethylation of the 5'-flanking region of the <i>IL-4</i> promoter	Sustains high levels of IL-4 secretion from Th2 cells	91
<i>IL-4</i>	Methylation of the 3' end of the <i>IL-4</i> locus	Promotes differentiation of precursor Th cells into Th 1 cells	91
<i>IFN</i>	Methylation of a AP-1– binding site in the proximal promoter resulted in reduced CREB and ATF2/c-Jun binding to this site	Associated with the loss of gene expression and the establishment of a Th2 polarization phenotype	94–96
<i>IL-13, IL-5</i>	Increased histone acetylation	Increases Th2-associated cytokine expression	73
<i>IFN</i> , <i>CXCL10</i>	Increased histone acetylation	Inhibits Th1-associated recall responses and expression of these cytokines	97
<i>FoxP3+</i>	Class II histone deacetylase inhibitors	Increase FoxP3+ expression and enhance the suppressive function of Foxp3+ regulatory T cells on Th2 response	99
<i>HLA-G</i>	miR-148a, miR-148b, and miR-152	Target a single nucleotide polymorphism at the 3' untranslated region of the gene	105
<i>IL-13</i>	miR-21, miR-1	Overexpressed in IL-13 transgenic mouse	106
<i>IL-12p35</i>	miR-21	Down-regulates gene expression in mouse inflamed airway	106
<i>TGF</i>	miR-146a	May mediate TGF β plus cytomix-induced apoptosis	107
<i>POU domain class 2 associating factor 1</i>	miR-126	Increases expression of the transcription factor	108

Table 2

Environmental factors known to lead to epigenetic changes that influence the asthma phenotype

Environmental factor(s)	Epigenetic effects	Relevance to asthma	References
Tobacco smoke	Suppresses HDAC2 expression and overall HDAC activity in macrophages	Enhances the expression of inflammatory mediators (GM-CSF, IL-8, IL-1 , TNF-)	112
Tobacco smoke	Induces hypermethylation of the promoter of <i>p16</i> ; <i>CYP1A1</i> , <i>RASSF1A</i> , <i>FHIT</i> in lung cancer cells	Relevance in asthma unknown	113–118
Tobacco smoke	Induces MAOB promoter hypomethylation in peripheral blood mononuclear cells	May serve as a biomarker of smoking-induced asthma	119
Maternal tobacco smoke	Induces global DNA hypomethylation (<i>AluYb8</i> but not <i>LINE1</i>) and <i>AXL</i> and <i>PTPRO</i> promoter hypermethylation in children	May serve as biomarkers of <i>in utero</i> exposure	122
BaP	Induces hypomethylation of a number of genomic repeats and sequence- specific hypo- and hyper- methylation changes in breast cancer cells	Relevance to asthma unclear	133
BaP	Induces H3K9-acetylation at the genome levels leading to hypo- and hyperacetylation in genes belonging to networks regulating gene expression, DNA replication and repair, and carcinogenesis (including <i>ATRX</i> , <i>MBD2</i> , <i>MBD3</i> , <i>HDAC1</i> , and <i>MTA3</i>)	Relevance to asthma not known	135
BaP	Decreases global DNA methylation, inhibits DNA methyltransferases <i>in vitro</i> , and interferes with recruitment of methylation machinery	May affect expression of asthma-related genes	136–139
Maternal PAH exposure from traffic pollution	Increased maternal exposure associated with increased hypermethylation of the <i>ACSL3</i> promoter in umbilical cord blood DNA of offspring	Hypermethylation of <i>ACSL3</i> promoter in umbilical cord blood associated with increased asthma risk in childhood	140
Oxidants	Post-translationally modifies the HDACs and creates HAT/HDAC stoichiometry imbalance	Contributes to the enhancement of IL-1 -stimulated inflammatory cytokine production (e.g., IL-8, IL-6, CXCL1, CXCL2, and CXCL3) in the inflamed airways	154,–155
LPS	May be a miRNA-146a target	Contributes to LPS priming	161
LPS	Drives Toll-like receptor signaling via Akt1-regulated expression of let-7e and miR-155	Contributes to macrophage hypersensitivity and endotoxin tolerance	162
Inhaled DEP	induces hypermethylation at specific CpGs of the <i>IFN-</i> promoter and hypomethylation at the <i>IL-4</i> promoter in splenic CD4+ cells	Hypersensitizes mice to intranasal <i>Aspergillus fumigatus</i> exposure	165
PM (10)	increases HAT activity and acetylated histone 4; remodels the <i>IL-8</i> promoter; action mediated via the induction of oxidative stress	Increases IL-8 expression and release from human alveolar basal epithelial cells	167
Exposure of elderly to ambient black carbon, but not PM2.5, for 4–7 days	Induces hypomethylation of <i>LINE-1</i>	May exacerbate asthma in this population	168
Methyl donors and coenzymes	Affects DNA methyltransferase activities and prevents aberrant global hypomethylation of the genome	Deficiencies in methyl donors predisposes complex diseases including asthma	170,171
Maternal diet rich in methyl donors	Favors lymphocyte maturation into a Th2 phenotype	Increases the risk of developing allergic airway disease in the offspring	172
Maternal folic acid supplementation	Increases the risk of wheeze and lower respiratory tract infections in progeny up to 18 months of age	Explains developmental reprogramming of asthma risk	173

Environmental factor(s)	Epigenetic effects	Relevance to asthma	References
Dust mite antigens	induces expression of miRNA-126 and activates TLR4	Increases inflammation, a Th 2 response, airway hyper- responsiveness via suppression of GATA-3	108