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Air Pollution and Epigenetics: Recent Findings

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Abstract The health effects of ambient air pollution are well-described, but less is known about the biological mechanisms mediating these health effects. In recent years, the hypothesis that epigenetics may play a role in driving exposure-disease associations has gained traction, in part because epigenetic modifications are labile and may respond to environmental exposures in ways that directly affect gene transcription and disease risk. The purpose of this review is to provide an understanding of the latest evidence to support this hypothesis. The studies selected for this review were collected through a PubMed search for articles published between January 2010 and July 2013 using the following epigenetics keywords: epigenetics, histone, microRNA, and DNA methylation; along with air pollutant keywords: air pollution, benzo[a]pyrene, nitrogen dioxide, ozone, particulate matter 1.0 (PM_1), particulate matter 2.5 ($PM_{2.5}$), particulate matter 10 (PM_{10}) and polycyclic aromatic hydrocarbon (PAH). A total of 38 original research articles were identified for our review. The scientific studies to date provide evidence that PAHs and $PM_{2.5}$ may have modest effects on methylation levels of certain CpG sites within candidate genes of interest in cardiovascular and respiratory disease as well as cancer. However, the data remain too sparse to draw any meaningful conclusions with regard to histone modifications, miRNAs, or effects of other pollutants such as NO_2, O_3, and SO_4.

Keywords Air pollution · Polycyclic aromatic hydrocarbons (PAHs) · Benzo[*a*]pyrene (B[a]P) · Particulate matter (PM) · DNA methylation · Long interspersed nuclear element-1 (LINE-1) · Histones · miRNA · Epigenetics

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Introduction

Ambient air pollution is associated with numerous adverse health outcomes, the most notable of which are allergic, respiratory, and cardiovascular diseases [1–3]. The biological mechanisms driving these exposure-disease associations are thought to be largely through oxidative stress or inflammatory pathways or endothelial or autonomic dysfunction [4]. Importantly, genetic variation in genes involved in these biological pathways can alter an individual's susceptibility to air pollution health effects [5], providing further evidence for their involvement. In recent years, the notion that epigenetics may play a role in driving exposure-disease associations has emerged, adding to the complexity of teasing out causal associations in population-based studies of air pollution.

Epigenetics – the study of alterations to gene function that do not change the DNA sequence itself – provides an attractive target when investigating the biological mechanisms through which air pollution might act. Alterations to epigenetic patterns have been implicated in numerous diseases [6–10], but most extensively in cancer [11, 12]. CpG methylation, histone modifications, and non-coding RNAs are all types of epigenetic marks that are labile [13] and thus may respond to environmental exposures in ways that directly affect gene transcription and disease risk (Fig. 1).

The purpose of this review is to summarize the latest scientific evidence supporting or refuting the hypothesis that ambient air pollutants can alter epigenetic patterns. We focus primarily on epidemiologic studies in the past three years, as several good reviews exist summarizing the scientific evidence prior to 2010 (Table 1) [14–17]. We include discussion of supporting experimental data when appropriate, although an exhaustive review of experimental data is beyond the scope of this review. The air pollutants investigated are polycyclic aromatic hydrocarbons (PAHs), including Benzo[*a*]pyrene (B[a]P), particulate matter (PM), ozone (O_3), nitrogen dioxide (NO_2), and

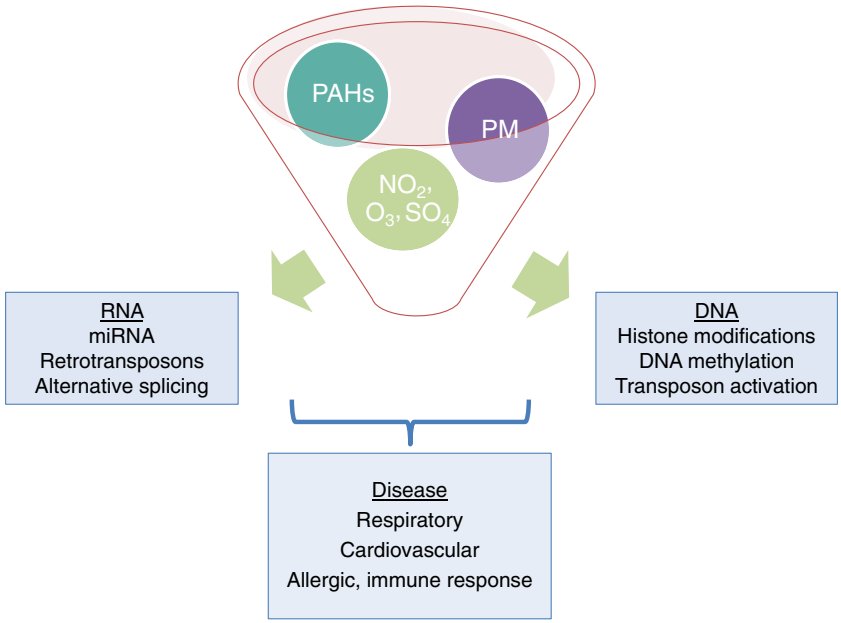

Fig. 1 Overview of mechanisms through which air pollutants may act on the epigenome and subsequent disease risk

sulfates (SO_4). The majority of data available investigates the effects of these pollutants on global DNA methylation or gene-specific CpG methylation, with limited investigations on microRNAs and histone modifications. The studies selected for this review were collected through a PubMed search for articles published between January 2010 and July 2013 using the following epigenetics keywords: epigenetics, histone, microRNA, and DNA methylation; along with air pollutant keywords: air pollution, benzo[a]pyrene, nitrogen dioxide, ozone, particulate matter 1.0 (PM_1), particulate matter 2.5 ($PM_{2.5}$), particulate matter 10 (PM_{10}), and PAH. A total of 38 original research articles were identified for our review.

PAH, B[a]P and DNA Methylation

PAHs are a byproduct of incomplete fossil fuel combustion or burning of organic elements from a variety of sources [18–20]. PAHs are ubiquitous environmental pollutants that are classified as Group 1 carcinogens by the International

Table 1 Summary of review articles on air pollution and epigenetics published prior to 2011

First Author, year	Years covered	Exposures included	Epigenetic metric evaluated	Conclusions
Bollati, V. 2010	2000-2009	vinclozolin, BPA, diet, PM10, benzene	DNA methylation, histone modification, LINE-1, Alu repeats	Environmental toxicants can modify epigenetic marks, some of which can be transmitted trans-generationally in animal models.
Durham, AL. 2011	2000-2010	diet, stress, prenatal tobacco smoke, benzene, ozone, diesel exhaust particles, PM10	DNA methylation, histone modification	Epigenetics help explain asthma development and inheritance, and provide a mechanism for understanding environmental influences on the disease.
Edwards, TM. 2007	≤2006	diet, cadmium, PAH	chromatin structure, DNA methylation, LINE-1	Environmental exposures influence many molecular mechanisms, including epigenetic regulation.
Jardim, MJ. 2011	≤2011	various air pollutants, DEP, ETS	miRNA, DNA methylation	Highlights a need for more research to understand how miRNAs respond to air pollutants to cause changes in disease processes.

DEP: diesel exhaust particle; ETS: environmental tobacco smoke; BPA: bisphenol-A; PM10: particulate matter < 10μml, PAH: polycyclic aromatic hydrocarbon

Agency for Research on Cancer (IARC) [21, 22]. When metabolized, PAHs can form DNA adducts and induce global hypomethylation and hypermethylation of specific genes associated with increased risk of neural impairments, neoplasia, and carcinogenesis [18, 20, 23, 24]. Epigenetic modifications are also increasingly linked to processes related to cardiovascular disease, atherosclerosis, and endothelial function [25]. Within the PAH mixtures, B[a]P is considered one of the most potent carcinogens [20, 24] and is often used as a tracer of PAH mixtures in studies to observe the health effects on humans and animals.

PAHs are associated with DNA methylation levels of repeated elements as well as with changes in levels of gene-specific DNA methylation (Table 2). Of the repeated elements, the long interspersed nucleotide element-1 (LINE-1) and the short interspersed nucleotide element (Alu) are the most-studied in epidemiological studies. In normal tissues, LINE–1 is typically highly methylated [26], whereas hypomethylation of LINE-1 is commonly observed in cancerous tissues and is believed to affect widespread alterations in gene expression and chromatin packaging control as well as higher genomic instability [27]. Demethylation of LINE-1 and Alu can increase their activity as transposable sequences, which may induce genomic alterations associated with unfavorable chronic health outcomes [26, 28]. Such epigenetic alterations are increasingly recognized as potential important mediators of health effects in the context of environmental or occupational settings that have high levels of PAHs. Several occupational studies, including studies of coke oven workers and steel, oil refinery, and petrochemical complexes, have observed associations between high levels of PAH exposure and LINE-1 hypomethylation [21, 22, 27].

Gene-specific DNA methylation has also been investigated, largely by taking a candidate-gene approach. In a study of smoke exposure among firefighters compared to non-firefighters, the firefighters had a lower level of methylation in dual specificity phosphatase 22-promoter (DUSP22), a gene suspected of playing a key role in inflammatory and proliferative disorders. In vitro experiments that challenged cell lines with B[a]P showed similar results, lending support to the evidence that B[a]P itself, not other constituents in smoke, can cause DNA methylation changes [30]. In a study of coke oven workers, PAH exposure was associated with hypomethylation in the promoters of O6-methylguanine-DNA methyltransferase (MGMT), p53, and interleukin-6 (IL-6) in workers of the largest steel, oil refinery, and petrochemical complex in southeastern Asia (Rayong, Thailand) [27]. These genes are involved in DNA repair, cell cycle arrest and cell death, and inflammation, respectively [21, 22, 27, 28]. Glutathione S-transferase Mu 2 (GSTM2) is another important gene in detoxification of PAHs. Tang et al investigated GSTM2 methylation in lung cancer cell lines and tissue samples, and found that aberrant hypermethylation of GSTM2 down-regulated GSTM2 expression and that tumor tissues had low or absent levels of GSTM2 expression, whereas adjacent non-malignant lung tissues had high levels of GSTM2 [31]. Lastly, one study of children in the Czech Republic evaluated effects of living in communities with high versus low ambient air pollution, including B[a]P levels that were sixfold higher in the highly polluted area, on DNA methylation using the Illumina Human Methylation 27k BeadChip [32]. The authors identified 58 CpG loci in different genes for which the difference in methylation level was greater than 10 %. In all loci, the DNA methylation level was lower in the highly polluted community. B[a]P-induced epigenetic effects have also been associated with immunosuppression, teratogenicity, and hormonal changes [20]. The preponderance of evidence to date suggests that PAH – or specifically B[a]P – exposure leads to hypomethylation in the promoters of genes involved in oxidative stress, inflammation, DNA repair, and cell cycle regulation, suggesting general up-regulation of these gene pathways that may have negative consequences for health.

Conversely, B[a]P exposure that causes DNA hypermethylation of tumor suppressor genes leads to their silencing in many human cancers. In vitro experiments of esophageal cancer cell lines treated with benzo[a]pyrene diol epoxide (BPDE) demonstrate that BPDE induces methylation of the retinoic acid receptor beta2 (RAR-β2) gene promoter [33]. RAR-β2 expression is suppressed in premalignant and malignant esophageal cells, and is associated with lung carcinogenesis [33]. Hypermethylation of HIC1, a tumor suppressor gene that is inactivated by DNA methylation in cancer tissues such as lung cancer, has also been observed [27].

While all of the above studies were conducted in adults, previous research suggests that early developmental periods are particularly vulnerable to epigenetic dysregulation in response to environmental exposures [18, 34]. Both human and animal models of prenatal exposure provide evidence that increased DNA adducts and global hypomethylation induce genomic and chromosomal instability [18, 34]. Herbtsman et al found prenatal exposure to PAHs was associated with lower global DNA methylation levels measured in umbilical cord white blood cell DNA [18]. Similarly, an animal study of zebrafish embryos focusing on early exposure to high concentrations of B[a]P observed significant global loss of DNA methylation and decreased DNA methylation in the germ cell-specific vasa promoter, whose altered gene expression can compromise developmental success and affect fertility [35, 36]. Taken together, the studies to date support the hypothesis that PAH exposure affects DNA methylation levels both globally and in specific genes involved in an array of disease processes.

Particulate Matter and DNA Methylation

The majority of human studies of PM and DNA methylation (Table 3) have evaluated $PM_{2.5}$. $PM_{2.5}$ has been inconsistently

Table 2 Highlights from articles describing associations between PAH, BaP, and DNA methylation

First Author, year	Design	Population	Age range	Exposure Assessment	Methylation measurement	N	Effect estimate, relative risk	Covariates
Duan H, 2013	Occupational cohort study	Coke oven workers, China	NA	PAHs	LINE-1, MGMT	144	LINE-1 and MGMT methylation levels were significantly lowered in PAH exposed workers. LINE-1, MGMT, and its hot CpG site-specific methylation were negatively correlated with urinary 1-hydroxypyrene levels (r=-0.329, p<0.001; r=-0.164, p=0.049 and r=-0.176, p=0.034, respectively).	age and sex
Fang X, 2013	Experimental	zebrafish	embryos	BaP	Global DNA Methylation, vasa, rassf1, tert, c-jun, and c-myca	30	2.4 μg/L effect on global methylation: β=-25.0 % (p=0.04) and on vasa β=-17.2 % (p<0.05); 24 μg/L effect β=-44.8 % , (p=0.02)	NA
Herbstman JB, 2012	Birth Cohort	Northern Manhattan Mothers and Newborns Study, New York City	newborn	PAHs	cord blood global methylation (log-transformed)	164	effect of PAH exposure (log-transformed): β=–0.11 (95 % CI: –0.21, 0.00)	maternal age, ethnicity, marital status, education, annual household income, child's sex, and mother's parity.
Ouyang B, 2012	Occupational cohort study	firefighters	23-53	smoke from firefighting	GSTP1, IFN-, RAD21, and DUSP22	38	median DUSP22 methylation in FF (15 %) and non-FF (27 %) differed (p=0.03). DUSP22 hypomethylation correlated with firefighting service years (r=-0.48, P=0.04)	age
Pavanello S, 2010	Occupational cohort study	Coke oven workers, Poland	20-59	PAHs	LINE-1, ALU, and p53	92	DNA methylation levels (global and p53 promoter methylation changes) were significantly different between workers and controls	sex, ethnicity, age, years of work, urinary 1-pyrenol
Peluso M, 2012	Occupational cohort Study	Coke oven workers, Rayong, Thailand	Mean age: 33.9	PAHs, compared coke oven worker, residents near the complex, and rural residents.	LINE-1, Alu, p53, p16, HIC1, O6-methylguanine, MGMT, IL-6	132	Workers, compared with rural residents, showed lower LINE-1 (74.8 % vs 78.0 %; P<0.001), p53 (8.0 % vs 15.7 %; P<0.001) and IL-6 methylation (39.2 % vs 45.0 %; P=0.027) and higher HIC1 methylation (22.2 % vs 15.3 %, P<0.001)	Age, gender, smoking, and percent neutrophils
Rossnerova A, 2013	Cohort study	Children from two regions in the Czech Republic	7-15	BaP, high vs low air pollution	Illumina 27K array	400	58 CpG loci in different genes for which the difference in methylation level was greater than 10 %.	Age, gender, asthma status
Tang SC, 2010	Case-control Study, Experimental	Patients with stage I or II non-small-cell lung	median: 67 yrs	BaP	GSTM2	50	GST-M2 mRNA expression is dependent on 5-Aza (10 μM) treatment (p<0.01). GST-M2	Age, gender, histology, stage of cancer

Table 2 (continued)

First Author, year	Design	Population	Age range	Exposure Assessment	Methylation measurement	N	Effect estimate, relative risk	Covariates
		carcinoma, Taiwan					overexpression protects against B[a]P-induced DNA damage.	
Ye F, 2010	Experimental	Esophageal squamous cancer cell lines	NA	BPDE	RAR-beta2	NA	BPDE induced methylation of the RAR-β2 gene promoter and recruited DNMT3A, but not DNMT3B, to the promoter. 5-Aza and DNMT3A small hairpin RNA (shRNA) vector antagonized the effects of BPDE on RAR-β2 expression.	NA

associated with DNA methylation in repeated elements, such as LINE-1 and Alu, or with other assays of global methylation. In an occupational cohort of boilermakers, personal $PM_{2.5}$ measured over the work shift was not associated with LINE-1 or Alu methylation measured in blood [37]. However, short-term black carbon (BC) exposure was associated with hypomethylation in Alu [26], and workplace industrial exposure from a steel, oil refinery, and petrochemical complex was associated with LINE-1 hypomethylation [27]. $PM_{2.5}$ during pregnancy, and particularly during the first trimester, was associated with global DNA hypomethylation in placental tissue [34]. DNA methylation in LINE-1 or Alu may play another role as well, which is to increase individual susceptibility to health effects of air pollution. At least one study to date has shown that, while pollutants such as black carbon, NO_2, CO, $PM_{2.5,}$ and sulfates were associated with endothelial adhesion molecules ICAM-1 and VCAM-1 blood levels, the effect was stronger among subjects with higher Alu or lower LINE-1 methylation status [29]. Given the scant number of studies, the various pollutants evaluated, and the likelihood of different PM constituents across these occupational and environmental settings, the impact of particulate matter on repeated element methylation or global DNA methylation remains uncertain.

Investigations of $PM_{2.5}$ exposure and DNA methylation have also focused on candidate genes of interest, primarily in the context of cardiovascular or respiratory disease. For example, several studies have evaluated the effect of PM on regulation of the inducible nitric oxide gene (iNOS), a gene responsible for production of nitric oxide, which is important for both cardiovascular and respiratory health. Recently, chronic exposure to $PM_{2.5}$ generated from welding activities was associated with a modest change in DNA methylation of the iNOS gene [37]. This work builds upon earlier evidence from three other cohorts suggesting that $PM_{2.5}$ can affect DNA methylation in iNOS [23, 38, 39•, 40]. In a cohort of steelworkers, 3 days of work exposure was associated with a decrease in iNOS promoter methylation [40]. In a cohort of elderly men, acute exposure to BC and $PM_{2.5}$ was associated with a decrease in iNOS methylation [23]. Similarly, in our own study of a cohort of children from Southern California, increased levels of $PM_{2.5}$ and PM_{10} were associated with decreased methylation in the iNOS promoter but increased methylation within the CpG island [38]. We also observed higher DNA methylation in endothelial nitric oxide synthase (eNOS). Moreover, the regulation of nitric oxide is quite complex, and we demonstrated that iNOS promoter haplotypes, in conjunction with PM exposure and DNA methylation levels, synergistically affected exhaled nitric oxide levels [39•]. The evidence to date, which spans acute and chronic exposures from occupationally and environmentally exposed subjects both young and old, consistently demonstrates that PM is associated with iNOS promoter methylation and affects production of nitric oxide. The extent that these modest effects

Table 3 Highlights from articles describing associations between particulate matter and DNA methylation

First Author, year	Design	Population	Age range	Exposure Assessment	Methylation measurement	N	Effect estimate, relative risk	Covariates
Bellavia A, 2013	Cross-over trial	Healthy adult volunteers	18-60	Concentrated ambient particles (CAPs)	LINE-1, ALU, TLR4, IL-12, IL-6, iNOS	15	Alu: β=-0.74, P=0.03; TLR4: β=-0.27, p=0.04	random intercept for subject, CpG and random slope for each position. Further adjusted for order of exposure
Bind MA, 2012	Cohort	Normative Aging Study	62-85	PM2.5, BC, SO4, CO, O3, NO2	LINE-1, ALU, F3, TLR-2, and ICAM-1	704	Effect modification of pollutants on fibrinogen, ICAM, VCAM, and CRP levels by methylation status	age, seasonality, body mass index (BMI), temperature, relative humidity, smoking status, statin use, diabetes and seasonality
Breton CV, 2012	Cohort	Children's Health Study	9-11	PM10, PM2.5	NOS1, NOS2A, NOS3	940	PM2.5: estimates ranged from-0.06 %(-0.18 - 0.06) to -1.0 % (-1.61, -0.56) for NOS2A promoter; and 0.33 % (0.01, 0.65) for NOS2A CpG island; and 2.8 % (1.77, 3.75) for NOS3	age, sex, race/ethnicity, experimental plate (for Pyrosequencing reactions), town of residence, asthma, month of DNA collection, and parental education.
Janssen BG, 2013	Birth cohort	Environage	Newborn	PM2.5	global DNA methylation	240	-2.13 % (-3.71, -0.54 %) per 5 μg/m3 increase in PM2.5	newborn's gender, maternal age , pre-gestational BMI, net weight gain, maternal education, newborn's ethnicity, smoking status , prenatal alcohol use, prenatal acetaminophen, delivery method, gestational age, parity, season at conception and trimester-specific apparent temperature.
Kile ML, 2013	Cohort Study	Boilermaker welders	21-61	PM2.5	LINE-1, ALU, iNOS	38	iNOS: β=0.25, p=0.04; Alu: β=0.05, p=0.47; LINE-1 β=−0.12, p=0.28	DNA methylation in the sample preshift, currently smoking, age, and wearing respirator
Kohli A, 2012	Cross-sectional study	Fresno and Stanford, CA	7–18	High vs low ambient air pollution (AAP)	IFN-γ , Foxp3 in T cells	102	Effect of AAP on IFN-γ: 1.90 (1.62, 2.23), AAP+SHS on Foxp3: 5.89 (5.45, 6.47)	age and gender
Madrigano J, 2011	Cohort	Normative Aging Study	21–80	PM 2.5, black carbon, SO4	LINE1, ALU	706	1 (IQR) increase in BC: β=-0.31 % (- 0.12- -0.50 %) in Alu. An IQR increase in SO4: β=- 0.27 % -(0.02- -0.52 %) in LINE-1.	season, temperature, a composite index of human discomfort due to combined heat and high humidity, age, smoking, BMI, prescription medication, alcohol intake, laboratory batch, and percent lymphocytes and percent neutrophils.
Madrigano J, 2012	Cohort	Normative Aging Study	21–80	PM 2.5, black carbon	iNOS, GCR	699	For a 10 mug/m(3) increase in PM.25 β=-0.6 % (-0.03, -1.1) and for a 1-mug/m(3) increase in BC β=-0.9 % (0.4, 1.4) for iNOS	baseline age, season, and time trend, apparent temperature, percent lymphocytes, percent neutrophils, body mass index, diabetes mellitus, and hypertension medication
Nadeau K, 2010	Cross-sectional study	Fresno and Stanford, CA	6-11	High vs low ambient air pollution, PAHs	Foxp3	181	Children with and without asthma in Fresno had significantly higher levels of methylation at the Foxp3 locus (P≤.05) compared to those in Stanford. PAH level correlated with methylation.	age and sex

Table 3 (continued)

First Author, year	Design	Population	Age range	Exposure Assessment	Methylation measurement	N	Effect estimate, relative risk	Covariates
Peluso M, 2012	Occupational cohort Study	Coke oven workers, Rayong, Thailand	Mean age: 33.9	Compared coke oven worker, residents near the complex, and rural residents.	LINE-1, Alu, p53, p16, HIC1, O6-methylguanine, MGMT, IL-6	132	Workers, compared with rural residents, showed lower LINE-1 (74.8 % vs 78.0 %; P<0.001), p53 (8.0 % vs 15.7 %; P<0.001) and IL-6 methylation (39.2 % vs 45.0 %; P=0.027) and higher HIC1 methylation (22.2 % vs 15.3 %, P<0.001)	Age, gender, smoking, and percent neutrophils
Salam MT, 2012	Cohort Study	Children's Health Study	6-11	PM2.5, PM10, NO2, O3	iNOS	940	PM2.5: β=-0.30 (-0.54, -0.06); PM10 β=-0.07 (-0.22, 0.08); NO2 β=0.10 (-0.25, 0.45); O3 β=-0.02 (-0.35, 0.32); NOS2 promoter haplotypes were globally associated with NOS2 promoter methylation (P=6.2×10(-8)).	Age, sex, ethnicity, asthma, respiratory allergy, parental education, community of residence, month of FeNO collection, NOS2 promoter haplotypes, and experimental plate
Soberanes S, 2012	Experimental (mice)	Male mice	6 - 8 week-old	Concentrated ambient particles (CAPs)	DNMT1, p16	6	PM 2.5 exposure increased expression of the DNMT1 2-fold, and p16 methylation 1-2 fold (p<0.01)	NA
Sofer T, 2013	Cohort Study	Normative Aging Study	56-88	Black carbon, SO4	31 asthma candidate genes	141	black carbon (p=0.05) and SO4 (p=0.02) were significantly associated with the methylation pattern in the asthma pathway	age and smoking
Tarantini L, 2009	Occupational cohort Study	Steel plant workers, Brescia, Italy	27–55	PM10	LINE1, ALU, iNOS	63	PM10: (β=-0.19 %; p=0.04) for Alu and (β=-0.34 %5mC; p=0.04) for LINE-1; no changes with post-exposure compared to baseline; post-exposure for iNOS: (β=-0.61 %5mC; p=0.02)	age, BMI, smoking, and number of cigarettes/day
Tarantini L, 2013	Occupational cohort Study	Steel plant workers, Brescia, Italy	27–55	PM10, PM1, zinc, iron	NOS3, EDN1	63	NOS3 methylation was negatively associated with PM10 (β=−0.2, 95 % CI −0.4 to −0.03), PM1 (β=−0.8, 95 % CI −1.4 to −0.1), zinc (β=−0.9, 95 % CI −1.4 to −0.3) and iron (β=−0.7, 95 % CI −1.4 to −0.01) exposures. Zinc exposure was negatively associated with EDN1 (β=−0.3, 95 % CI −0.8 to −0.1) methylation.	age, BMI, current smoking status, non-steroidal anti-inflammatory drugs and per cent monocytes in the differential blood count.

alter cardiovascular and respiratory health is still an active area of investigation.

Other candidate genes of interest are those that may affect asthma pathogenesis. In one study that took a pathway-based approach, exposure to BC was significantly associated with the methylation patterns in 31 genes previously identified as part of the asthma pathway [41•]. These pollutant exposures had the greatest effect on methylation in genes that were either part of the Th2/B-cell signaling pathway or contributed to controlling eosinophils and airway inflammation, obstruction, and hyper-responsiveness [41•]. Increased exposure to ambient air pollution, comparing communities with high and low levels of air pollutants, is associated with hypermethylation of the Forkhead box transcription factor 3 (Foxp3), a key transcription factor in regulatory T- cells [42•]. These changes are hypothesized to lead to impaired Treg-cell function and increased asthma morbidity. A further study in the same population demonstrated similar effects of ambient air pollution on hypermethylation of interferon-gamma (IFN-gamma), another gene important to asthma, in effector T cells [43].

There have been very few human exposure trials of air pollution for the purpose of investigating DNA methylation. However, in one small crossover trial of exposure to concentrated ambient particles (CAPs), 15 healthy adult participants were exposed for 130 minutes to fine CAPs, coarse CAPs, or HEPA-filtered medical air [28]. Fine CAPs exposure lowered Alu methylation, whereas coarse CAPs exposure lowered Toll-like receptor 4 (TLR4) methylation, another gene of interest in asthma pathogenesis. Interestingly, hypomethylation in these loci was associated with increased blood pressure, providing some of the first evidence that methylation in certain genes may mediate PM exposures on blood pressure [28].

A handful of studies have also evaluated air pollution effects on genes involved in cancer pathogenesis. In vivo and in vitro experiments demonstrate that direct exposure to $PM_{2.5}$ can induce methylation of the p16 promoter, a tumor suppressor gene important in cancer in mice and in epithelial cell lines [44]. Expression of DNA methyltransferase 1 (DNMT1) was also elevated in these studies, suggesting that increased DNMT1 may be the mechanism through which the promoter is methylated.

In some cases, DNA methylation may alter individual susceptibility to pollutant exposures as opposed to being directly affected by the exposure. For instance, the effects of several pollutants with ICAM-1 and VCAM-1 blood levels were stronger among subjects having higher tissue factor (F3) or Toll-like receptor 2 (TLR-2) methylation status [29].

Not all candidate genes demonstrate associations. DNA methylation in the glucocorticoid receptor (GCR), a gene implicated in stress response, as well as in interleukins 6 and 12 (IL-6, IL-12) genes involved in immune response, have not been associated with $PM_{2.5}$ to date [23, 28]. Nevertheless, there is emerging literature that suggests $PM_{2.5}$ may affect DNA methylation in certain genes involved in asthma pathogenesis. These effects are complicated by the knowledge that the DNA methylation levels can vary by cell type, and therefore the response to pollutant exposure may also vary.

The data on PM other than $PM_{2.5}$ are sparse. A handful of studies from one cohort evaluated PM_{10} or PM_1 [45, 46]. Short-term exposure to metal-rich PM in an occupational cohort of steel workers was associated with leukocyte telomere length – an inflammatory and cardiovascular risk factor. However, no association was observed with DNA methylation level in the telomerase catalytic enzyme gene (hTERT), the gene responsible for telomere length [45]. Additional studies in the same cohort provide evidence that PM exposure may also affect blood coagulation through altered methylation [46]. PM_{10} and PM_1, as well as zinc and iron exposures, were associated with decreased eNOS methylation, whereas only zinc exposure was associated with endothelin-1 (EDN1) methylation. This hypomethylation in both eNOS and EDN1 mediated the PM-induced coagulation effects as measured by the endogenous thrombin potential [46].

Other Air Pollutants and DNA Methylation

Very few studies have investigated the effects of other ambient pollutants, such as O_3, NO_2, or SO_4, on epigenetic modification. No associations have been reported for O_3 thus far [39•]. One study in asthmatic children found that NO_2 exposure in combination with high levels of DNA methylation, the beta-2 adrenergic receptor (ADRB2) gene, was associated with severe asthma [47]. ADRB2 is a gene expressed on airway smooth muscles important in reducing inflammatory cytokine production, stimulating relaxation, and clearing lung fluid. SO_4 was associated with decreased LINE-1 methylation in a cohort of elderly men [26]. Additionally, in the same cohort, the effects of SO_4 on fibrinogen were modified by LINE-1 methylation level, whereas the effects of NO_2 on ICAM-1 and VCAM-1 blood levels were stronger in subjects with higher Alu and lower LINE-1 methylation [29]. SO_4 has also been linked to altered methylation levels in several genes in the asthma pathway, and these are largely different genes than those associated with BC [41•].

Air Pollutants and miRNAs

MicroRNAs (miRNAs) are highly conserved noncoding small RNAs that regulate the expression of broad gene networks at the posttranscriptional level. Although there is debate over whether to classify miRNAs as epigenetic marks, we have chosen to include them in this review for the following reasons. Over 1,000 miRNAs have been discovered, and they regulate nearly two-thirds of human genes [48]. Because miRNAs

control numerous cellular processes, they may be critical for mediating biological responses to air pollutants [17]. To this end, a few studies have begun investigating the effects of air pollution exposure on selected miRNAs of interest.

In one of the first studies to evaluate miRNA response, human airway epithelial cells were challenged with diesel exhaust particles (DEP) for 24 hours, and expression changes were measured using a miRNA microarray [49•]. Jardim et al found 197 detectable miRNAs that were either up- or down-regulated in response to exposure, of which the top 12 were primarily associated with inflammatory and tumorigenic processes [49•]. Candidate miRNAs putatively involved in oxidative stress and inflammation – specifically miR-222, miR-21, and miR-146a – were subsequently investigated in a cohort of steelworkers [50]. Pre- and post- workday exposures to PM1, coarse PM, and PM metal components (chromium, lead, cadmium, arsenic, nickel, manganese) were evaluated, and miR-222 and miR-146a expression were correlated with lead exposure, although none were correlated with PM size fractions [50]. Polymorphisms in miRNA-processing genes were also found to modify the effects of ambient pollution on cardiovascular disease biomarkers [51]. Lastly, a recent study comparing subjects living in an area contaminated with electronic waste to those living in an unpolluted area found a total of 182 differentially expressed miRNAs [52]. The contaminated area likely contained high levels of heavy metals, atmospheric polychlorodibenzo-p-dioxins (PCDDs), polychlorodibenzofurans (PCDFs), and polybrominated diphenyl ether (PBDE), along with thousands of other pollutants.

While the emerging literature on the associations between ambient air pollutants and miRNAs is nascent, this avenue of investigation holds promise, considering that small changes to a single miRNA have the potential to affect broad downstream gene networks involved in multiple disease processes.

Air Pollutants and Histones

Histones are proteins that form one of the fundamental building blocks in chromatin organization. Modification of histones can therefore affect chromatin organization as well as gene regulation [53]. Examples of such modifications include lysine acetylation, lysine methylation, arginine methylation, serine phosphorylation, lysine ubiquitination, and many others [54, 55]. Histone modifications provide yet another potential mechanism by which air pollution exposure may affect downstream regulation of genes and gene pathways. However, due primarily to the laborious nature of the laboratory assays and the associated costs, measurement of histone modifications in large population studies has not been feasible.

To date, limited information exists on the effect of ambient air pollutants on histone modifications. In one human study, long-term exposure to inhalable nickel, arsenic, and iron was associated with increased histone 3 lysine 4 dimethylation (H3K4Me2) and histone 3 lysine 9 acetylation (H3K9Ac) on histones from blood leukocytes [56]. In an animal study, neonatal exposure to B[a]P decreased the acetylation of H3K14 in the steroidogenic acute regulatory protein (StAR), an important protein in testosterone production [57]. B[a]P damaged testosterone production and sperm counts in these rats, plausibly as the result of a direct effect on epigenetic regulation of StAR. In HeLa cells challenged with B[a]P, histone H3 trimethylated at lysine 4 (H3K4Me3) and H3K9Ac were enriched, whereas the association of DNA methyltransferase-1 (DNMT1) with the L1 promoter was reduced [24]. Cytosine methylation within the L1 promoter CpG island was also reduced. Pharmacological inhibition altered these BaP-mediated histone epigenetic modifications [24]. Lastly, experiments using knockout mice demonstrated that Sirt1, a member of class III histone deacetylase, controls lung inflammation and coagulation after PM exposure. PM exposure aggravated lung vascular leakage and inflammation in Sirt1 knockout mice, and these effects were correlated with increased NF-kappaB acetylation and activation [58].

Conclusion

The last several years have produced an emerging set literature on the hypothesis that epigenetic modifications are susceptible to air pollution exposure, both during pregnancy and in childhood or adulthood. For histone modifications and miRNAs, the data are still much too sparse to make any meaningful conclusions. The same is true for many of the specific air pollutants, namely NO_2, O_3, and SO_4. However, it is becoming clear that PAHs and $PM_{2.5}$ may have modest effects on methylation levels of certain CpG sites within candidate genes of interest in cardiovascular and respiratory disease as well as cancer. The application of epigenome-wide approaches, such as DNA methylation array platforms and bisulfite-sequencing, as they become more widely used and the results publicly available will undoubtedly shed additional light on these associations and discover new ones. Moreover, the extent to which data from large-scale methylation studies are shared for common exposures such as PAH and $PM_{2.5}$ holds the promise of maximizing the potential inferences.

In all likelihood, many of the candidate gene associations observed today are some of the more robust associations, plausible biomarkers of exposure. They may not be as sensitive to the cell type used or timing of exposure, and thus they have been observed despite potential confounding by cell type in mixed blood. As an example, the consistent associations observed for $PM_{2.5}$ and iNOS have been found using buccal cell DNA in children as well as whole blood in adults. As the field evolves and we improve sensitivity of laboratory assays, develop advanced statistical methods specific for

analyzing epigenetic data, and integrate information across genotype, epigenotype, and exposure, we will undoubtedly gain further insights, although likely raising as many questions as are answered.

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Compliance with Ethics Guidelines

Conflict of Interest Carrie V. Breton and Amy N. Marutani declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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