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ENVIRONMENTAL EPIGENETICS (A BACCARELLI AND A CARDENAS, SECTION EDITORS)

# The Impact of Air Pollution on Our Epigenome: How Far Is the Evidence? (A Systematic Review)

Rossella Alfano[1] · Zdenko Herceg[2] · Tim S. Nawrot[1,3] · Marc Chadeau-Hyam[4] · Akram Ghantous[2] · Michelle Plusquin[1]

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## Abstract

**Purpose of Review** This systematic review evaluated existing evidence linking air pollution exposure in humans to major epigenetic mechanisms: DNA methylation, microRNAs, long noncoding RNAs, and chromatin regulation.

**Recent Findings** Eighty-two manuscripts were eligible, most of which were observational (85%), conducted in adults (66%) and based on DNA methylation (79%).

**Summary** Most observational studies, except panel, demonstrated modest effects of air pollution on the methylome. Panel and experimental studies revealed a relatively large number of significant methylome alterations, though based on smaller sample sizes. Particulate matter levels were positively associated in several studies with global or LINE-1 hypomethylation, a hallmark of several diseases, and with decondensed chromatin structure. Several air pollution species altered the DNA methylation clock, inducing accelerated biological aging. The causal nature of identified associations is not clear, however, especially that most originate from countries with low air pollution levels. Existing evidence, gaps, and perspectives are highlighted herein.

**Keywords** Air pollution · Epigenetics · DNA methylation · MicroRNAs · Noncoding RNA · Chromatin

## Introduction

Ambient air pollution is among the leading risk factors for mortality. Components such as airborne particles smaller than 2.5 μM in aerodynamic diameter ($PM_{2.5}$) were estimated to cause 4.2 million deaths representing 7.6% of total global deaths in 2015 [1]. According to the World Health Organization (WHO), 92% of the population breathes air with unhealthy levels of pollutants [2]. Pollution is closely linked to climate changes, and future levels of air pollution will depend on emissions and global climate changes [3].

Since air pollution is a complex mixture, we still have little understanding of the individual contribution of its different components [4]. Components of air pollution include but are not limited to particulate matter (PM), ozone ($O_3$), sulfur dioxide ($SO_2$), nitrogen oxides ($NO_x$), carbon monoxide (CO),

Akram Ghantous and Michelle Plusquin contributed equally to this work.

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✉ Akram Ghantous
GhantousA@iarc.fr

✉ Michelle Plusquin
michelle.plusquin@uhasselt.be

[1] Centre for Environmental Sciences, Hasselt University, Hasselt, Belgium

[2] Epigenetics Group, International Agency for Research on Cancer (IARC), 150 Cours Albert-Thomas, 69008 Lyon, France

[3] Environment & Health Unit, Leuven University, Leuven, Belgium

[4] Department of Epidemiology and Biostatistics, The School of Public Health, Imperial College London, London, UK

benzene, black carbon, polycyclic aromatic hydrocarbons, and toxic metals. Because of their small size, airborne particles can be inhaled deeply into the lungs and deposited in the alveoli, and the smallest particles can directly reach the bloodstream [5]. Exposure to air pollution increases the risk for cardiovascular [6, 7] as well as respiratory diseases [8] and cancer [9]. In fact, air pollution has been classified by the International Agency for Research on Cancer as a group I carcinogen [10]. Besides this, evidence is mounting that exposure to air pollution is associated with neuro- and cognitive developmental alterations [11] in newborns [12] and children [13–15]. Oxidative stress, immune and inflammation responses, and mitochondrial changes are commonly considered as putative mechanisms underlying these health outcomes [7]. One of the mechanisms through which air pollution exposure can induce these biomolecular changes involves DNA damage and epigenetic alterations, which can influence health outcomes across life stages and possibly across generations [16, 17••, 18].

Epigenetics is the science of nongenetic mitotically heritable changes that result in variations in gene expression [19, 20]. Four major interacting systems ensure epigenetic control of gene expression: DNA methylation, histone modification, noncoding RNAs, and chromatin remodeling. These communicating mechanisms ensure the somatically heritable states of gene expression [21]. Epigenetic states display plasticity and are subjected to intrinsic (e.g., age, sex, genetic polymorphisms) and extrinsic (e.g., environmental exposures and dietary habits) influences [22].

Few reviews (all nonsystematic) are available on air pollution and epigenetics, and important novel technological developments in exposure assessment and in epigenome-wide association (EWAS) studies have become available since then. Accordingly, we provide a systematic review analyzing the existing evidence on the associations between air pollution and the major levels of human epigenetic control: DNA methylation, histone modifications, microRNA (miRNA), and long noncoding RNA (lncRNA) and chromatin regulation. We are only just beginning to understand the multitude of effects of air pollution on epigenetics, and this work could provide a timely guide into this rapidly evolving field.

## Methods

This systematic review was conducted according to the STROBE guidelines [23]. The search strategy used to identify epidemiological studies examining the association between air pollution and epigenetic changes was made up of four stages, following the PRISMA statement guidelines [24], as depicted in Fig. 1.

In the first stage, articles were identified through a literature search. First, the search was performed through PubMed and Scopus engines without any time restriction and using the MeSH terms "air pollution" and "epigenomics" and the keywords: "particulate matter," "ultrafine particle*," "$PM_{2.5}$," "$PM_{10}$," "black carbon," "elemental* carbon," "nitrogen* dioxide*," "nitrogen* oxide*," "$NO_x$," "$NO_2$," "PAH" and "polycyclic aromatic hydrocarbon*," "histon*," "DNA methylation," "DNA hydroxymethylation," "non-coding RNA," "miRNA," "chromatin remodelling," "chromatin," "chip-on-chip," and "chip seq." Boolean operators were used to create every possible term combinations as reported in the Online Resource 1. The last search was run on the 6th September 2018.

The second stage consisted of the screening of all the papers identified. Two researchers (R.A. and M.P.) independently reviewed the relevance of the records through consultation of titles and abstracts (and in case uncertainty assessing the full texts).

In the third stage, the full text of the records was examined for eligibility. Eligibility criteria used were the following: (1) the paper is written in English, (2) the paper is an original article, (3) the paper deals with human species, (4) the paper deals with in vivo study, (5) the paper examines any epigenetics mechanism, and (6) the paper studies ambient air pollution as main exposure or the effects of air pollution through an experimental design.

In the fourth stage, selected studies were grouped according to epigenetics mechanisms under investigation and study population; the following information was extracted: authors, relevant country, study design and period, population size, age, and cohort from which data were derived, air pollution exposures examined, epigenetic markers analyzed along with the method of detection, confounders identified, main findings, and risk estimates.

For DNA methylation and hydroxymethylation, separate tables for adults and children are presented.

## Results

We have analyzed existing evidence associating air pollution to the major levels of human epigenetic control (Fig. 1): DNA methylation (Tables 1, 2, and 3), noncoding RNAs (Table 4), and chromatin regulation (Table 4). Characteristics of the studies included in the review are summarized in Fig. 2.

### DNA Methylation

DNA methylation was the most widely investigated epigenetic mechanism in response to air pollution (Fig. 2b). We have separated the results into those observed in children (Table 1) and those in adults (Tables 2 and 3). All studies among children were observational (cohort, cross-sectional, case-control, and longitudinal; Table 1), while adult studies were either

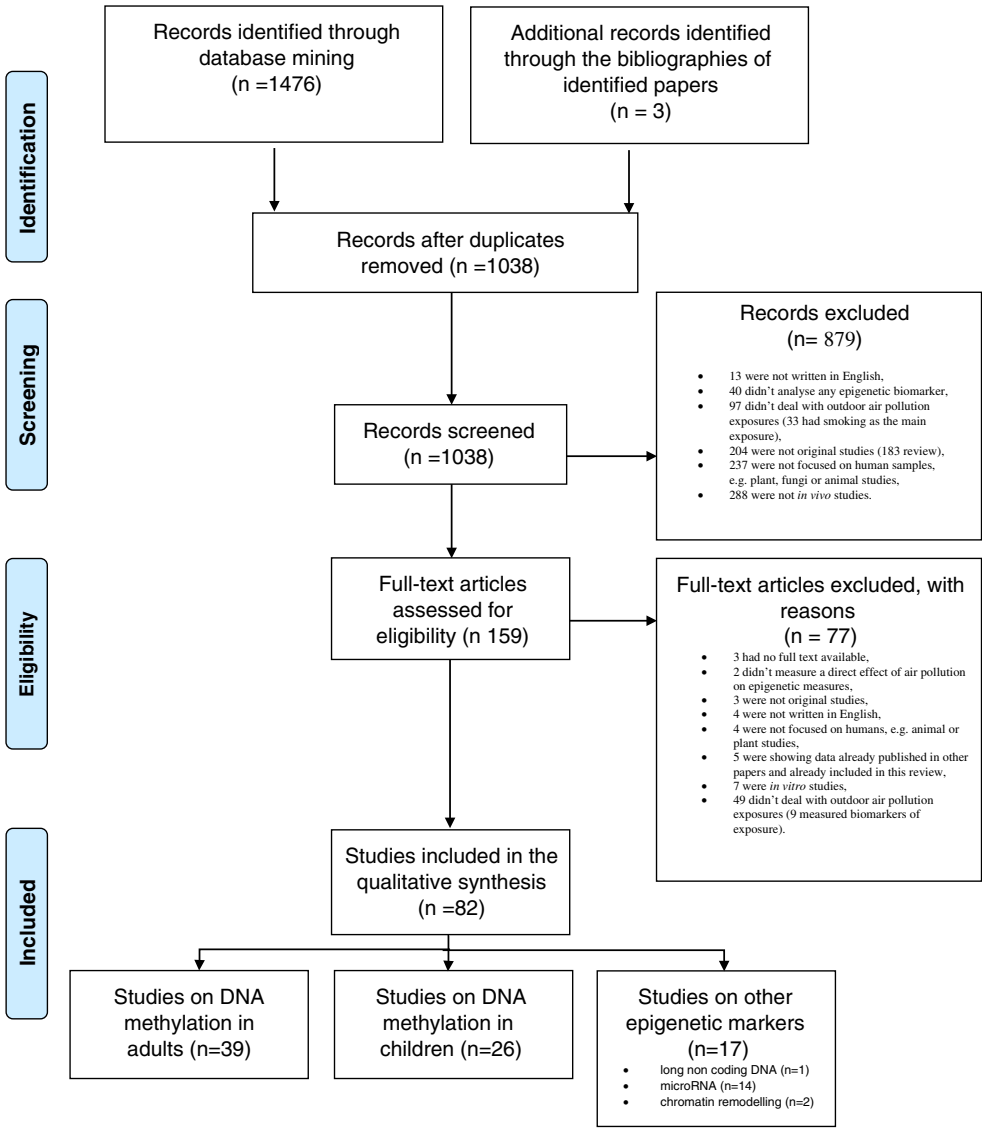


**Fig. 1** Flow diagram, following the PRISMA statement guidelines, of the search strategy used to identify studies examining the association between air pollution and epigenetic changes

observational (cohort, cross-sectional, case-control, longitudinal, and panel; Table 2) or experimental (crossover; Table 3).

In children (Table 1), EWAS studies in cohort or longitudinal studies showed little effect of perinatal air pollution exposure on the newborn's blood epigenome (nos. 1–4). The few genes for which differential methylation did seem to relate to air pollution encompass *EOGT* and *COLEC11* in response to PM and three mitochondria-related genes (*LONP1*, *HIBADH*, and *SLC25A28*) and *ADOR2B* in relation to $NO_2$. Two other studies used an EWAS approach but in a cross-sectional design, comparing blood from individuals living in high- vs. low-polluted areas (nos. 5–6). The first (no. 5 in South Africa) showed no significant results but was based on a relatively small sample size ($n = 21$ newborns), while the second (no. 6 in Czech Republic) reported 9916 significant CpG sites, but half of its participants were asthmatics (which the study attempted to correct for). Both studies were conducted in countries with comparable air pollution burdens [20–30 μg/m$^3$ of particles of less than 10 μM in aerodynamic diameter ($PM_{10}$), according to WHO Global Urban Ambient Air Pollution 2016 Database http://www.who.int/phe/health_topics/outdoorair/databases/cities/en/]. All other studies were performed in countries that exhibit < 20 μg/m$^3$ of $PM_{10}$ and are considered relatively lowly polluted, with the exception of one study in Mexico (20 μg/m$^3$ of $PM_{10}$; no. 14), two in China (54 μg/m$^3$ of $PM_{10}$; nos. 10 and 15), and one in Iran (60 μg/m$^3$ of $PM_{10}$; no. 7). However, the Mexican and Chinese studies used targeted methylation approaches, among which long interspersed nucleotide elements (LINE-1 or L1) were used as a proxy for global methylation levels. The Mexican study did not find a significant association between L1 methylation and overall $PM_{10}$, but a positive relationship was observed with one of the $PM_{10}$ components, benzo[*b*]fluoranthene (no. 14). All other studies (including the Chinese) investigating the relationship between PM and L1 methylation reported a negative association (nos. 15 and 18), while ozone

**Table 1** The characteristics of the 26 studies included in the present systematic review investigating DNA methylation and air pollution in children. Articles result from a search carried out in April 2018 using a combination of MeSH terms and keywords in two different search engines (PubMed and Scopus) and have been selected using specific inclusion criteria derived from the PECO statement. For each study, the table reports the first author and the year of publication along with the reference, the relevant country, the study design, the study period, the population size, age and cohort from which data were derived, the air pollution exposures examined, the epigenetic biomarkers analyzed along with the method of detection, the confounders identified, the main findings, and the risk estimates. Studies using untargeted exploration of the methylome are presented first followed by those using targeted approaches

| No. | Authors, year | Country | Study design | Study period | Population, age | Study name from which data are derived |
|---|---|---|---|---|---|---|
| 1 | Abraham et al., 2018 [25] | France | Cohort study | 2003 onward | 668 mother-child pairs, mean gestational age 39.8 weeks | EDEN |
| 2 | Plusquin et al., 2018 [17] | Belgium, Spain, Greece, UK, Italy | Cohort meta-analysis and longitudinal study | 1990 onward | 197 newborns from ENVIRONAGE, 84 from INMA, 99 from RHEA, and 75 from RHEA, at birth; 780 children from ALSPAC, at birth, 7 and 15 years | ALSPAC, ENVIRONAGE, INMA, RHEA, and Piccolipiù |
| 3 | Gruzieva et al., 2017 [26] | Spain, France, Netherlands, USA, Norway | Cohort meta-analysis | MeDALL (1994–1997, 2003–2007)Generation R (2002–2006)CHS (1995–1997)MoBa (1999–2008) | 1508 newborns from MeDALL, Generation R, CHS, and MoBa cohorts, at birth | MeDALL, Generation R, CHS, and MoBa |
| 4 | Breton et al., 2016 [27] | USA | Cohort study | 2003 | 240 children, age range 10–13 years; additional 280 children were included in the replication study | CHS |
| 5 | Goodrich et al., 2016 [28] | South Africa | Cross-sectional study | 2010–2011 | 21 newborns, mean gestation age 38.8 weeks | MACE |
| 6 | Rossnerova et al., 2013 [29] | Czech Republic | Cross-sectional study | 2010 | 200 children (100 asthmatics and 100 controls), mean age 12 years | |
| 7 | Maghbooli et al., 2018 [30] | Iran | Nested case-control study | 2016–2017 | 100 mother-child pairs distinguished in 2 groups according to residence in polluted ($N = 50$) and nonpolluted ($N = 50$) areas, mean gestational age 38 weeks | |
| 8 | Janssen et al., 2013 [31] | Belgium | Cohort study | 2010–2012 | 240 mother-newborn pairs, at birth | ENVIRONAGE |
| 9 | Herbstman et al., 2012 [32] | USA | Nested cohort of asthmatics and healthy urban children | 1998 onward | 164 African American and Dominic newborns, at birth | CCCEH |
| 10 | Li et al., 2018 [33] | China | Case-control study | 2013 | 105 children affected by allergic rhinitis and 90 control subjects, mean age 8.5 and 8.9 years, respectively | |
| 11 | Nawrot et al., 2018 [34] | Belgium | Cohort study | 2010–2014 | 407 newborns, mean age 39.3 weeks | ENVIRONAGE |
| 12 | Neven et al., 2018 [35] | Belgium | Cohort study | 2010–2014 | 500 placenta samples from 814 pairs of mothers and neonates, mean gestational age 39.2 weeks | ENVIRONAGE |
| 13 | Prunicki et al., 2018 [36] | USA | Cohort study | 2010–2015 | 188 children (121 asthmatic and 67 healthy), mean age 14.7 years | |
| 14 | Alvarado-Cruz et al., 2017 [37] | Mexico | Cross-sectional study | 2010 | 150 school children, age range 7–10 years | |
| 15 | Cai et al., 2017 [38] | China | Cross-sectional study | 2011–2013 | 181 (80 fetal growth restriction and 101 normal) newborns, mean gestational age 38.07 weeks | |
| 16 | Lovinsky-Desir et al., 2017 [39] | USA | Nested cohort of asthmatics and healthy urban children | 2012–2015 | 135 African American and Dominic newborns, age range 9–14 years | CCCEH |
| 17 | Saenen et al., 2017 [40] | Belgium | Cohort study | 2010–2013 | 361 mother-newborn pairs, mean gestational age 39.3 weeks | ENVIRONAGE |
| 18 | Breton et al., 2016 [41] | USA | Cohort study | 2002 onward | 392 children, mean age 11.2 | CHS |
| 19 | Somineni et al., 2016 [42] | USA | Cross-sectional study | | 70 siblings (35 asthmatic and 35 healthy), mean age 11 years | ESS |
| 20 | Hew et al., 2015 [43] | USA | Cross-sectional study | | | FACES |

**Table 1** (continued)

| No. | Authors, year | Country | Study design | Study period | Population, age | Study name from which data are derived |
|---|---|---|---|---|---|---|
| | | | | | 256 children (171 nonasthmatic and asthmatic), age range 10–21 years | |
| 21 | Janssen et al., 2015 [31] | Belgium | Cohort study | 2010 onward | 381 mother-newborn pairs, mean gestational age 39.2 weeks | ENVIRONAGE |
| 22 | Breton et al., 2012 [44] | USA | Cohort study | 2002 onward | 940 children, mean age 9.3 years | CHS |
| 23 | Salam et al., 2012 [45] | USA | Cohort study | 2004–2007 | 940 children, mean age 9.3 years | CHS |
| 24 | Tang et al., 2012 [46] | USA | Nested cohort of asthmatics and healthy urban children | | 53 African American and Dominic newborns, at birth | CCCEH |
| 25 | Nadeau et al., 2010 [47] | USA | Cross-sectional study | | 181 children divided into: Fresno Asthma Group (FA), *n* 71; Fresno Non Asthmatic Group (FNA), *n* 30; Stanford Stanford Asthma Group (SA), *n* 40; and Stanford Non Asthmatic Group (SNA), *n* 40; median age 14, 13, 12, and 12.5 years, respectively | FACES |
| 26 | Perera et al., 2009 [48] | USA | Nested cohort of asthmatics and healthy urban children | 1998 onward | 20 African American and Dominic newborns for the discovery plus the full subset of 56 children for main analyses, at birth | CCCEH |

| Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| $NO_2$ and $PM_{10}$ hourly concentrations were modeled at the women's home addresses during pregnancy using the ADMS to estimate exposure at days 1, 2, and 3 before delivery, 1 week before delivery, 1 month before delivery, each trimester of pregnancy, and the whole pregnancy | Epigenome-wide methylation[a], methylation at L1 and *Alu* regions and at candidate placental genes, in placenta samples | Illumina Infinium Human Methylation 450K array | Child sex, parity, maternal age at end of education, season of conception, study center, BMI, maternal age at delivery, maternal smoking during pregnancy and gestational duration, batch, plate, chip, estimated cell-type proportions, temperature and humidity | $PM_{10}$ exposure the day before birth was positively associated with *Alu* methylation. The candidate approach revealed 9 CpGs significantly associated with $NO_2$ and $PM_{10}$ exposure. Only 2 of these associations (located on *ADORA2B* gene) with $NO_2$ during the second trimester and the whole pregnancy remained significant also in the EWAS. |
| Prenatal and early life (at 7 and 15–17 years of age) residential $PM_{10}$ exposure were estimated via the dispersion model (ALSPAC), LUR (INMA, RHEA and Piccolipiù), and combination of dispersion and LUR model (ENVIRONAGE) | Epigenome-wide DNA methylation[a], in cord and peripheral blood samples from 7- and 15-year-old children | Illumina Infinium Human Methylation 450K array | Sex of the child, maternal smoking during pregnancy, blood cell composition | No methylome-wide significant association between maternal $PM_{10}$ exposure during pregnancy and cord blood DNA methylation in the meta-analysis was observed. The CpG site cg21785536 (*EOGT*) was negatively associated with pregnancy and annual (at 7 and 15–17 years of age) residential $PM_{10}$ exposure in a longitudinal model integrating the 3 studied age groups of the ALSPAC cohort (− 1.2% per 10 μg/$m^3$; raw *p* value = 3.82e-8). Pathway analyses on the corresponding 100 strongest genes of the longitudinal model revealed enriched pathways relating to the GABAergic synapse, p53 signaling and to NOTCH1. |

**Table 1** (continued)

| Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| $NO_2$ during pregnancy was estimated at maternal home addresses through LUR | Epigenome-wide DNA methylation[a] and CpG selection, in cord blood at birth and in blood samples in childhood, respectively | Illumina Infinium Human Methylation 450K array | Sex, maternal smoking during pregnancy, municipality at birth (in BAMSE), cohort-specific batch indicator(s), cohort indicator (in the pooled MeDALL sample set), and ancestry (in CHS), age (for analysis in children) and cell composition | Maternal $NO_2$ exposure during pregnancy was significantly associated with DNA methylation in newborns for 3 CpG sites in mitochondria-related genes: cg12283362 (*LONP1*), cg24172570 (*HIBADH*), and cg08973675 (*SLC25A28*). Only the association on *SLC25A28* gene was found significant also in children samples. |
| Estimates of prenatal air pollution exposures for $PM_{2.5}$ and $PM_{10}$ were interpolated on residential address reported on the birth certificate and at the time of the baseline questionnaire using inverse-distance-squared weighting | Epigenome-wide methylation[a] (only probes mapped to the promoter region were included in the analysis yielding 178,309 loci), in newborn blood spots | Illumina Infinium Human Methylation 450K array | Gender, plate, and cell types | 31 loci were associated with either $PM_{10}$ or $PM_{2.5}$ using FDR-corrected $p$ values of less than 0.15. Two loci were evaluated for replication in a separate population of 280 CHS subjects of which 1 successfully replicated (*COLEC11*, cg03579365). |
| Maternal residence in a heavily industrialized and polluted region (south Durban) or in a lesser polluted area (north Durban) | Epigenome-wide methylation[a], in cord blood | Illumina Infinium Human Methylation 450K array | Maternal HIV status, gestational age, and sex | No significant DMS was observed with an FDR level of 10%. Top DMS were more likely to be hypomethylated and located in CpG islands and pathway analysis via LR path revealed significantly enriched gene related to xenobiotic metabolism, oxygen and gas transport, and sensory perception of chemical stimuli. |
| Residence in highly polluted region (Ostrava) and a control region (Prachatice) | Epigenome-wide methylation[a], in blood samples | Illumina Infinium Human Methylation 27K array | Gender, age of the children, birthweight, the gestation and the breastfeed length, smoking | 9916 CpG sites were significantly different methylated between children from highly polluted region vs. control region from which 58 CpG sites had differences > 10%. The methylation of all these 58 CpG sites was lower in children from the polluted region. |
| Regional background daily levels of $PM_{2.5}$ and $PM_{10}$ during the pregnancy at the mother's address were calculated from measurements at 9 monitoring stations | Global DNA methylation[a], in placenta samples | HPLC | Age, sex, and region | A positive significant association was detected between $PM_{2.5}$ and $PM_{10}$ in the first trimester and placental global DNA methylation levels. |
| $PM_{2.5}$ exposure during pregnancy was estimated using a spatial temporal interpolation method (kriging) | Global DNA methylation levels[a], in placenta samples | UPLC/MS-MS | Newborn's gender, maternal age, gestational age, parity, maternal education, smoking status, prenatal acetaminophen use, season at conception, and trimester-specific apparent temperature | Placental global DNA methylation was negatively associated with $PM_{2.5}$ exposures during the whole pregnancy and relatively decreased by 2.19% (95% CI − 3.65, − 0.73%; $p = 0.004$) for each 5 $\mu g/m^3$ increase in exposure to $PM_{2.5}$. This association was also confirmed in a multilag model. $PM_{2.5}$ exposure during the first and second trimesters was negatively associated with DNA global methylation, while no significant association was found for the third trimester. |
| Maternal total PAH, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, BaP, | Global DNA methylation[a], in cord blood | ELISA | Maternal age, ethnicity, marital status, education, annual household income, child's sex, and mother's parity | Prenatal total PAH exposure and pyrene were associated with lower global methylation in umbilical cord white blood cells (respectively for total PAH: $\beta = -0.11$, $p = 0.05$; for pyrene: $\beta = -0.18$ $p = 0.01$). |

**Table 1** (continued)

| Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| indeno[1,2,3-*cd*]pyrene, dibenz[*a,h*] anthracene, and benzo[*g,h,i*]perylene exposures were assessed from personal prenatal air monitoring during the third trimester of pregnancy | | | | |
| Individual exposure concentration of $PM_{10}$ and $PM_{2.5}$ over 1 year was assessed via the PM station near the participants' residence address by the kriging interpolation or estimated using activity-based dynamic exposures | DNA methylation of IFN-γ and IL-4 promoter, in blood samples | Bisulfite sequencing | Age, gender, exclusive breastfeeding within 4 months after birth, and parental history of allergic disease | $PM_{2.5}$ exposure level was positively correlated with methylation level in the IFN-γ promoter region ($\beta = 1.441$, SE = 0.672, $p = 0.039$). |
| $PM_{2.5}$ exposure levels during pregnancy at the maternal home address were estimated using a spatial temporal interpolation method in combination with a dispersion model | DNA methylation at CpG sites within the promoter regions of 8 circadian pathway genes (*CLOCK*, *BMAL1*, *NPAS2*, *CRY1–2*, and *PER1–3*), in placenta samples | Pyrosequencing | Newborn's sex, newborn's ethnicity, maternal age, maternal education, maternal smoking status, gestational duration, pre-pregnancy BMI, total weight gain during pregnancy, cord blood vitamin D, hour of delivery, time window-specific apparent temperature, and season at conception | Placental circadian pathway methylation was significantly increased with $PM_{2.5}$ exposure during the third trimester ($p < 0.0001$). Single-gene models showed relative methylation differences (log-fold change) in placental *NPAS2* (+0.16; $p = 0.001$), *CRY1* (+0.59; $p = 0.0023$), PER2 (+0.36; $p = 0.0005$), and *PER3* (+0.42; $p = 0.0008$) for an IQR increase (8.9 $\mu g/m^3$) in the third trimester $PM_{2.5}$ exposure. |
| Exposures to $PM_{2.5}$, black carbon, and $NO_2$ during the pregnancy at maternal residence address were estimated by the kriging interpolation | DNA methylation in the promoter genes of key DNA repair and tumor suppressor genes (*APEX1*, *OGG1*, *PARP1*, *ERCC1*, *ERCC4*, *p53*, and *DAPK1*), in placenta samples | Pyrosequencing | Neonate's sex, ethnicity, and parity and maternal age, education, smoking habits, and pre-pregnancy BMI, as well as gestational age, season at delivery, and batch effect | Promoter methylation was positively associated with $PM_{2.5}$ in *APEX1* (7.34%, 95% CI 0.52 to 14.16, $p = 0.009$), *OGG1* (13.06, 3.88 to 22.24, $p = 0.005$), *ERCC4* (16.31%, 5.43 to 27.18, $p = 0.01$), and *p53* (10.60%, 4.46 to 16.74, $p = 0.01$), whereas promoter methylation of *DAPK1* (−12.92%, −22.35 to −3.49, $p = 0.007$) was negatively associated with $PM_{2.5}$ exposure. Black carbon exposure was associated with elevated promoter methylation in *APEX1* (9.16%, 4.06 to 14.25, $p = 0.01$) and *ERCC4* (27.56%, 17.58 to 37.55, $p < 0.0001$). Promoter methylation was not associated with pollutant exposure in *PARP1* and *ERCC1*, and $NO_2$ exposure was not associated with methylation in any of the genes studied. |
| Short- and long-term exposures to CO, $O_3$, $NO_2$, and PM $_{2.5}$ were computed using inverse distance weighting of measurements from the air quality monitoring stations according to distance from the participant's residence | DMRs on *FOXP3* and *IL-10* genes, in blood samples | Pyrosequencing | Sex, age, and BMI, season and asthma status | Methylation of *FOXP3* was associated with exposure to air pollutants 90 days prior blood drawing and exactly to: CO (coef = 1.157, $p = 0.001$), $O_3$ (coef = −2.158, $p = 0.002$), $NO_2$ (coef = −1.504, $p = 0.002$), $PM_{2.5}$ (coef = 0.903, $p = 0.012$). |
| $PM_{10}$ and its components were monitored at the roof | L1 DNA methylation and probe by probe DNA methylation of 3 | Pyrosequencing | | Although overall $PM_{10}$ exposure did not show any significant association, individual $PM_{10}$ |

**Table 1** (continued)

| Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| of schools during the study period | repair genes from the BER pathway (*APEX1*, *OGG1*, and *PARP1*), in blood samples | | Gender, cotinine levels, BMI, dietary folate intake, biomass burning, time spent outdoors, and the proportion of WBC | components showed a significant positive relationship between L1 methylation and benzo[*b*]fluoranthene concentration. Similarly, acenaphthene, indeno[1,2,3-*cd*]pyrene, and pyrene were related to increased methylation in specific CpG dinucleotides in all the 3 genes under study, while vanadium was significantly related to the methylation in selected CpG sites in *APEX* and *PARP1* genes. |
| Trimester-specific $PM_{10}$ exposures during pregnancy were calculated by daily air pollution data obtained from the database of Wenzhou Municipal Environmental Bureau | DNA methylation of L1 and 2 genes (*HSD11B2* and *NR3C1*), in placenta samples | Pyrosequencing | Gestational age, infant gender, maternal age and education, pre-pregnancy BMI, delivery model, day of the week and day of the month for delivery, maternal passive smoking during pregnancy, $NO_2$ and $SO_2$ exposure levels, season and trimester-specific $PM_{10}$ | First trimester $PM_{10}$ exposure was negatively associated with placental L1 ($\beta = 1.78$, 95% CI −3.35, −0.22%), second trimester and whole pregnancy $PM_{10}$ exposure was directly associated with *HSD11B2* ($\beta = 1.42$, 95% CI 0.24, 2.57%; $\beta = 1.98$, 95% CI 0.53, 3.43%, respectively). Alterations were bigger considering only the fetal growth restriction group. |
| BC exposure was measured from personal monitoring at 24-h periods at the beginning and end of the week-long physical activity monitoring period | *FOXP3* DNA methylation, in buccal samples | Pyrosequencing | Age, sex, race/ethnicity, BMI *Z*-score, asthma, atopy (total IgE ≥ 80 IU/ml), SHS exposure, and heating season | Children with high personal BC exposure had higher *FOXP3* promoter 1 methylation compared to children with low BC (80.9 ± 4.8 vs. 79.1 ± 4.7, $p = 0.04$). |
| $PM_{2.5}$ exposure during pregnancy was estimated using a spatial temporal interpolation method (kriging) | *LEP* promoter DNA methylation, in placenta samples | Pyrosequencing | Maternal age, gestational age, and pre-pregnancy BMI, newborn sex, maternal education, smoking status, ethnicity of the newborn, and trimester-specific season | Placental *LEP* methylation was 1.4% lower (95% CI −2.7, −0.19%) in association with an interquartile range increment (7.5 μg/m$^3$) in second trimester $PM_{2.5}$ exposure. |
| Prenatal air pollution exposures for $O_3$, $NO_2$, $PM_{10}$, and $PM_{2.5}$ monitoring station air quality data were interpolated to the residence at birth | L1 and *AluYb8* methylation, in newborn blood spots | Pyrosequencing | Sex, ethnicity, cell types, mother's education level, in utero tobacco smoke, and plate effect (for L1 analyses only) | Prenatal exposure to multiple air pollutants in the first trimester of pregnancy was associated with lower DNA methylation in L1, whereas later exposure to $O_3$ was associated with higher L1 methylation levels. No significant associations were observed for *AluYb8*. |
| Current and birth (first year of life) elemental carbon attributable to traffic (ECAT) were estimated using a land-use regression model | DNA methylation at the cg23602092 site on the *TET1* promoter, in nasal epithelial samples | Pyrosequencing | Age and sex | In controls, cg23602092 methylation was associated with higher current ECAT values ($p < 0.001$), and a similar trend (but not significant) was shown in asthmatic children. No association was detected for birth ECAT. Replication in the Pediatric Environmental Exposure Study cohort of 186 children did not find significant association with neither current or birth ECAT. |
| PAHs averaged over 24-h (24 h), 1-week, 1-month, 3-month, 6-month, and 1-year time periods were estimated using LUR model | DNA methylation at 4 CpGs of *FOXP3* gene, in blood samples | Pyrosequencing | Analyses were stratified by asthma (or rhinitis) status | PAH exposure was significantly associated with increased *FOXP3* methylation. Significant associations were not observed until 1 month for asthmatic subjects and 3 months for nonasthmatic subjects. |
| Regional background levels of $PM_{2.5}$ during pregnancy were interpolated using a spatial | DNA methylation at CpG sites within specific regions of the mitochondrial genome (MT-RNR1 and D-loop), in placenta samples | Pyrosequencing | Gender, maternal age, gestational age, smoking, maternal education, parity, ethnicity, and season at conception | A positive association was found for mtDNA methylation (MT-RNR1 and D-loop, separately as well as combined) with $PM_{2.5}$ exposure during the entire gestation. The association was most |

**Table 1** (continued)

| Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| temporal interpolation method (kriging) | | | | pronounced in the first trimester of pregnancy (MT-RNR1: 1.27%, 95% CI 0.23 to 2.32%; D-loop 0.44%, 95% CI 0.12 to 0.75%; n D 356 for an IQR increment of $PM_{2.5}$ levels). |
| Cumulative average exposure levels 7 days, 1 month, 6 months, and 1 year before the date of DNA collection were calculated from 24-h average measurements of $PM_{10}$, and $PM_{2.5}$ at monitoring stations | DNA methylation at *NOS1*, *NOS2a* and *NOS3* genes, in buccal samples | Pyrosequencing | Age, sex, race/ethnicity, experimental plate, town of residence, month of DNA collection, parental education, asthma, allergy, and wheeze | A 5-μg/$m^3$ increase in $PM_{2.5}$ was associated with a 0.20% [95% confidence interval (CI) − 0.32, − 0.07] to 1.0% (95% CI − 1.61, − 0.56) lower DNA methylation at *NOS2A* position 1, 0.06% (95% CI − 0.18, 0.06) to 0.58% (95% CI − 1.13, − 0.02) lower methylation at position 2, and 0.34% (95% CI − 0.57, − 0.11) to 0.89% (95% CI − 1.57, − 0.21) lower methylation at position 3, depending on the length of exposure and CpG locus. One-year $PM_{2.5}$ exposure was associated with 0.33% (95% CI 0.01, 0.65) higher average DNA methylation of 4 loci in the *NOS2A* CpG island. A 5-μg/$m^3$ increase in 7-day and 1-year $PM_{2.5}$ was associated with 0.6% (95% CI 0.13, 0.99) and 2.8% (95% CI 1.77, 3.75) higher *NOS3* DNA methylation. No associations were observed for *NOS1*. $PM_{10}$ showed similar but weaker associations with DNA methylation in these genes. |
| Cumulative average exposure levels 7 days before the visit were calculated from 24-h average measurements of $PM_{10}$, $PM_{2.5}$, $NO_2$, and $O_3$ (10 am–6 pm) at monitoring stations | DNA methylation at *iNOS* gene, in buccal samples | Pyrosequencing | Age, sex, ethnicity, asthma, respiratory allergy, parental education, community of residence, month of FENO collection, NOS2 promoter haplotypes, and experimental plate | A 5-mg/$m^3$ increase in 7-day average $PM_{2.5}$ exposure was significantly associated with 0.30% lower *iNOS* methylation (p value = 0.01). No significant association was found for any other exposure. |
| Maternal PAH exposure was assessed from personal prenatal air monitoring during the third trimester | DNA methylation in promoter region of *IFN-γ* gene, in cord blood | Methylation sensitive restriction fingerprinting followed by PCR | Age at delivery, ethnicity, sex, exposure to tobacco smoke (ETS), and receipt of public assistance as dichotomous variables modeled dichotomously | Median percent methylation in *IFN-γ* region 1 was significantly higher among participants with high vs. low PAH exposure (97.1 vs. 88.7%, $p < 0.01$). This association was confirmed in the linear model adjusted for sex and spline age at delivery. Similarly, methylation of *IFN-γ* region 2 was significantly positively associated to maternal ln-PAH when modeled as a restricted cubic spline function, adjusted for ETS, public assistance, and spline age at delivery. Predicted values of percent methylation increased up to approximately 3.5 ng/$m^3$ PAH, after which they decreased. |
| Estimates of annual average PAH exposures were based on LUR analyses | DNA methylation of the *FOXP3* gene, in blood samples | Bisulfite sequencing PCR | | Increased methylation of the 8 CpG islands in the promoter region and of the 13 CpG islands in the intronic region was detected in the order of FA > FNA > SA > SNA groups. Moreover, a direct association was found between average daily PAH exposure in the FA group and the number of methylated CpG islands. |
| Maternal total PAH exposures were assessed from personal prenatal air monitoring during the | Discovery of de novo methylation sites through methylation sensitive restriction fingerprinting and DNA | Methylation sensitive restriction fingerprinting followed by PCR | Gender and ethnicity | 31 DNA sequences were identified whose methylation status was dependent on the level of maternal PAH exposure. Six sequences were found to be homologous to known genes: *ACSL3*, *RAD21*, |

**Table 1** (continued)

| Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| third trimester of pregnancy | methylation of the discovered candidate genes, in cord blood | | | *DUSP22*, *SFMBT2*, *SCD5*, and *WWOX*: As *ACSL3* exhibited the highest concordance between the extent of methylation and its expression in fetal tissues, it was the only gene chosen for further investigation. *ACSL3* was found to be significantly associated with maternal airborne PAH exposure exceeding 2.41 ng/m$^3$ (OR = 13.8; $p = 0.001$; sensitivity = 75%; specificity = 82%). |

*ADMS*: quasi-Gaussian Atmospheric Dispersion Modeling System; *ALSPAC*: Avon Longitudinal Study of Parents and Children; *CCCEH*: Columbia Center for Children's Environmental Health Cohort Study; *CHS*: Children's Health Study; *DMR*: differentially methylated region; *DMS*: differentially methylated site; *ENVIRONAGE*: Environmental Influences on Early Ageing Study; *ESS*: Exposure Sibling Study; *FACES*: Fresno Asthmatic Children's Environment Study; *INMA*: Environment and Childhood study; *L1*: LINE-1; *LUR*: land-use regression; *MACE*: Mother and Child Environmental study; *MeDALL*: Mechanisms of the Development of Allergy Study; *MoBa*: Norwegian Mother and Child Cohort Study

[a] Untargeted exploration of the methylome

showed the opposite trend (no. 18). No association was found for *Alu* methylation with different air pollutants from a study in 11-year-old children (no. 18), while a positive association was observed with $PM_{10}$ in placenta (no. 1). In contrast, global methylation was found always negatively associated with PAH, pyrene (no. 9) and PM (no. 8) levels, except from the Iranian study that found a positive association with PM (no. 7). Most of the studies investigated DNA modifications in relation to chronic exposure to air pollution, while only a few described effects of short-term exposure (nos. 1, 13, 14, 16, 20 and 23). Comparison of effects of short and chronic exposure to air pollution showed that the strength of associations was higher with chronic than recent exposure to air pollution (nos. 13, 20, and 22).

Similarly, in adult EWAS cohort studies (Table 2), few CpGs were significantly methylated (however, none could be replicated in different studies) in response to various air pollution components and based on blood as a surrogate tissue (nos. 1–11). Again, all these studies were conducted in relatively low-polluted countries (exhibiting < 20 μg/m$^3$ of PM based on the WHO Global Urban Ambient Air Pollution 2016 Database). China was the only country identified in the literature (nos. 13, 14, 16, 17, 23, and 25–27) that had relatively high pollution levels, but only targeted DNA methylation (i.e., not EWAS) analyses have been performed in this population. Even though EWAS analyses in adult cohort studies have so far identified only a limited number of significant CpGs, one observation seems to be consistent across three EWAS studies addressing epigenetic aging and conducted in two different continents (represented by Germany and USA); this finding is that various air pollution components contribute to accelerated aging (nos. 5, 9, and 10) calculated from DNA methylation levels according to the Horvath and Hannum methods (using respectively 353 and 71 chronological age-correlated CpGs) [103, 104].

Similar to studies among children, in adults, global methylation estimated from EWAS approaches (nos. 12 and 15) was negatively associated with overall PM levels (Table 2). Similarly, a negative association was found between PM and L1 methylation levels, while no association was found for methylation of *Alu* elements (no. 32). Negative associations with global or L1 methylation were reported for $NO_2$ (nos. 7 and 15), black carbon (nos. 22 and 32), and $SO_4$ (no. 22) (Table 2). DNA methylation changes have been described for exposure durations to air pollutants ranging from only a few hours (no. 24) to several years. Longer-term exposures yielded more significant associations (no. 15). Two studies also investigated global hydroxymethylation levels in relation to PM but reported conflicting findings (no. 12 vs. 14).

As for the experimental studies (Table 3), all were conducted in adults and were crossover intervention trials in which the volunteers were sequentially exposed to particulate or diesel exhaust mixtures and to controlled exposures (filtered air). Similar

**Table 2** The characteristics of the 32 observational studies included in the present systematic review investigating DNA methylation and air pollution in adults. Articles result from a search carried out in April 2018 using a combination of MeSH terms and keywords in two different search engines (PubMed and Scopus) and have been selected using specific inclusion criteria derived from the PECO statement. For each study, the table reports the first author and the year of publication along with the reference, the relevant country, the study design, the study period, the population size, age and cohort from which data were derived, the air pollution exposures examined, the epigenetic biomarkers analyzed along with the method of detection, the confounders identified, the main findings, and the risk estimates. Studies using untargeted exploration of the methylome are presented first followed by those using targeted approaches

| No. | Authors, year | Country | Study design | Study period | Population, age | Study name from which data are derived |
|---|---|---|---|---|---|---|
| 1 | Mostafavi et al., 2018 [49••] | Italy, Netherlands, Switzerland, and UK | Panel study | 2013–2015 | 157 healthy never-smoking adults, age range 50–70 years | |
| 2 | Lichtenfels et al., 2018 [50] | Netherlands | Cohort study | 2007–2013 | 1622 adults, mean age 47.3 y | LifeLines |
| 3 | Dai et al., 2017 [51] | USA | Cohort study | 1999–2013 | 646 elderly males, mean age 73.7 years | NAS |
| 4 | Fiorito et al., 2017 [7] | Italy | Case-control cardiovascular study nested in a cohort | 1993–2013 | 386 adults (193 affected by cardiovascular diseases vs 193 healthy controls), mean age 54 years | |
| 5 | Nwanaji-Enwerem et al., 2017 [52] | USA | Cohort study | 2000–2011 | 552 elderly males, mean age 74.7 years | NAS |
| 6 | Panni et al., 2017 [53••] | Germany, USA | Cohort meta-analysis | KORA F3 (2004–2005), KORA F4 (2006–2008), and the NAS (1999–2007) | 2956 participants including 500 subject from KORA F3 1799 from KORA F3 and 657 from NAS, mean age 53.1, 60.9, and 72.4 years, respectively | KORA F3, F4, and NAS |
| 7 | Plusquin et al., 2017 [54] | Italy and Belgium | Cohort meta-analysis | 1993–2006 | 454 and 159 subjects were included in the analyses for Italy and Belgium, mean age 54.2 and 58.8 years, respectively | EPIC Italy and EPIC NL |
| 8 | Chi et al., 2016 [55] | USA | Cohort study | 2000–2012 | 1207 participants, mean age 69.6 years | MESA |
| 9 | Nwanaji-Enwerem et al., 2016 [56] | USA | Cohort study | 2000 onward | 1032 elderly males, mean age 74.8 years | NAS |
| 10 | Ward-Caviness et al., 2016 [57] | Germany | Cohort study | 2006–2008 | 1777 subjects, mean age 61 years | KORA F4 |
| 11 | Carmona et al., 2014 [58] | USA | Cohort study | 1999 onward | 141 elderly men, range age 56–88 years | NAS |
| 12 | De Nys et al., 2018 [59] | Belgium | Panel study | 2015 | 26 healthy volunteers, median age 23 years (M), 22 years (F) | |
| 13 | Liu et al., 2017 [60] | China | Cohort meta-analysis | | 110 adults were recruited from Zhuhai cohort, 118 adults from Wuhan cohort, and 79 adults from Tianjin cohort, mean age 55, 51, and 65 years for Zhuhai, Wuhan, and Tianjin, respectively | |
| 14 | Sanchez-Guerra et al., 2015 [61] | China | Cross-sectional study | 2008 | 60 truck drivers and 60 indoor office workers, age range 18–46 years | BTDAS |
| 15 | De Prins et al., 2013 [62] | Belgium | Cross-sectional study | 2010 | 48 nons-moking adults (couples residing at the same address), median age 49 years | |
| 16 | Wang et al., 2018 [63] | China | Panel study | 2014–2015 | 36 healthy college students, mean age 24 years | |
| 17 | Wang et al., 2016 [64] | China | Panel study | 2014–2015 | 36 healthy college students, mean age 24 years | |
| 18 | Sofer et al., 2012 [65] | USA | Cohort study | | 92 elderly men | NAS |

**Table 2** (continued)

| No. | Authors, year | Country | Study design | Study period | Population, age | Study name from which data are derived |
|---|---|---|---|---|---|---|
| 19 | Peng et al., 2016 [66] | USA | Cohort study | 2000–2011 | 551 elderly males, mean age at first visit 73.3 years | NAS |
| 20 | Ouyang et al., 2012 [67] | USA | Cross-sectional study | | 38 adults including 18 professional firefighters (FF) and 20 controls with no previous professional firefighting experience (non-FF), age range 23–53 years | |
| 21 | Madrigano et al., 2012 [68] | USA | Cohort study | 1999–2009 | 735 elderly males, mean age 72 years | NAS |
| 22 | Madrigano et al., 2011 [69] | USA | Cohort study | 1999–2007 | 706 elderly males, mean age 72.2 years | NAS |
| 23 | Liu et al., 2008 [70] | China | Cross-sectional study | | 107 participants, mean age 57.2 years | |
| 24 | Lepeule et al., 2014 [71] | USA | Cohort study | 1999–2009 | 76 elderly men, mean age 76.3 years | NAS |
| 25 | Hou et al., 2014 [72] | China | Cross-sectional study | 2008 | 60 truck drivers and 60 indoor office workers, mean age 30.3 and 33.5 years | BTDAS |
| 26 | Guo et al., 2014 [73] | China | Cross-sectional study | 2008 | 60 truck drivers and 60 indoor office workers, mean age 30.3 and 33.5 years | BTDAS |
| 27 | Chen et al., 2015 [74] | China | Panel study | 2014 | 30 adults affected by chronic obstructive pulmonary disease, mean age 64 years | |
| 28 | Cantone et al., 2017 [75] | Italy | Cross-sectional study | 2010–2011 | 168 adult obese, mean age 50.7 | SPHERE |
| 29 | Callahan et al., 2018 [76] | USA | Population-based study of breast cancer | 1996–2001 | 588 adult females affected by breast cancer, age range 30–79 years | WEB |
| 30 | Bind et al., 2015 [77] | USA | Cohort study | 1999–2009 | 777 elderly males, mean age 74.8 years | NAS |
| 31 | Bind et al., 2014 [78] | USA | Cohort study | 1999–2009 | 777 elderly males, mean age 72 years | NAS |
| 32 | Baccarelli et al., 2009 [79] | USA | Cohort study | 1999–2007 | 718 elderly males, mean age 73.3 years | NAS |

| Air pollution exposures | Epigenetic biomarkers,tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| 24-h personal $PM_{2.5}$, $PM_{2.5}$ absorbance, and UFP exposure were monitored in 3 sessions over 1 year. The average of the 24-h personal air pollution measurements across the 3 sessions was used as a proxy for long-term average personal exposure. Long-term ambient exposure was estimated using LUR | Epigenome wide DNA methylation[a], in peripheral blood samples | Illumina Infinium Human Methylation 450K array | Age, sex, BMI, and blood cell composition | Personal exposure to $PM_{2.5}$ was associated with methylation changes at 13 CpG sites. 69 DMRs were associated with personal PM 2.5, 42 DMRs with personal PM 2.5 absorbance exposure, 16 DMRs with personal exposure to UFP, 4 DMRs with ambient exposure to $PM_{2.5}$, 16 DMRs with ambient $PM_{2.5}$ absorbance exposure, and 15 DMRs with ambient UFP exposure. |
| Long-term exposure (annual average) to $NO_2$, $PM_{10}$, and $PM_{2.5}$ was estimated using LUR model | Epigenome-wide DNA methylation[a], in blood samples | Illumina Infinium Human Methylation 450K array | Sex, age, BMI, current smoking, pack-years, technical covariates, and blood cell composition | $NO_2$ exposure (per 10 $\mu g/m^3$) was genome-wide significantly associated with differential DNA methylation at 7 CpG sites: negatively with cg04908668 (*PSMB9*), cg00344801 (*TTC38*), and cg02234653 (*AP1S3*); positively with cg14938677 (*ARF5*), cg18379295 (*GNG2*), cg25769469 (*PTCD2*), and cg08500171 (*BAT2*). No significant |

**Table 2** (continued)

| Air pollution exposures | Epigenetic biomarkers,tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| | | | | association was found for the other pollutants under investigation. Replication in the KORA F3 and KORA F4 cohorts failed. |
| One-year moving average concentrations of $PM_{2.5}$ species (Al, Ca, Cu, Fe, K, Na, Ni, S, Si, V, and Zn) were estimated from daily measurements at a stationary monitoring station | Epigenome-wide DNA methylation[a], in blood samples | Illumina Infinium Human Methylation 450K array | Total PM 2.5 mass, WBC, age, BMI, cigarette pack-years, smoking status, alcohol consumption, years of education, year and season and batch effect | 20 significant CpGs for Fe, 8 for Ni, and 1 for V. Particularly, cg10911913 on *SLFN11* gene was significant for all 3 metals. |
| Long-term annual average exposures to $NO_2$,$NO_x$, and $PM_{2.5}$ were estimated using a LUR model | Epigenome-wide[a] and functional region DNA methylation, in blood samples | Illumina Infinium Human Methylation 450K array | Sex, center of recruitment, season of pollutant measurements, BMI, smoking habits, alcohol intake, Mediterranean diet score, physical activity, educational level, prevalent diabetes, hypertension, and hyperlipidemia | No significant site was detected by EWAS. Looking at DNA methylation probes involved in 17 inflammatory pathways, 2 were associated with air pollution: "ROS/Glutathione/ Cytotoxic granules" ($PM_{2.5}$) and "Cytokine signaling" ($PM_{2.5}$ and $NO_2$). |
| 1-year $PM_{2.5}$ and the following major $PM_{2.5}$ component species (organic carbon (OC), elemental carbon (EC), sulfate, nitrate, and ammonium) were estimated using the GEOS-chem chemical transport model | (Hannum and Horvath) DNAm-age[a], in blood samples | Illumina Infinium Human Methylation 450K array | Chronological age, blood cell types, temperature, pack-years, smoking status, season, BMI, alcohol consumption, and education | An IQR increase in 1-year $PM_{2.5}$, sulfate, and ammonium exposures was significantly associated respectively with a 0.64-, 0.51-, and 0.63-year increase in Horvath DNAm-age ($p < 0.05$). |
| Short- and mid-term (− 1, − 7, and − 28 days) $PM_{2.5}$ ambient concentrations measured hourly at monitoring stations | Epigenome-wide DNA methylation[a], in blood samples | Illumina Infinium Human Methylation 450K array | Age, sex, average annual exposure the year before the visit, and white blood cell proportions | 12 CpG sites were associated with PM concentration (1 for 2-day average, 1 for 7-day, and 10 for 28-day) at a genome-wide Bonferroni significance level. Out of these 12 sites, 9 expressed increased methylation, 4 (annotated in *NSMAF*, *C1orf212*, *MSGN1*, *NXN*) showed $p > 0.05$ and $12 < 0.5$. |
| Yearly mean concentration of $NO_2$, $NO_x$, $PM_{2.5}$, $PM_{coarse}$, $PM_{10}$, and $PM_{2.5}$ absorbance (soot) estimated by land-use regression models | Global, functional region and epigenome-wide DNA methylation[a], in blood samples | Illumina Infinium Human Methylation 450K array | Position of the sample on the microarray, age, smoking status, and sex (except for EPIC-NL which has only females and never and former smokers), prospective cancer diagnosis later in life (only for EPIC Italy) and blood cell composition | Meta-analysis of global DNA methylation found exposure to $NO_2$ was significantly associated with a global hypomethylation, and both $NO_2$ and $NO_x$ were associated with hypomethylation of CpG island's shores and shelves and gene bodies. Meta-analysis of EWAS did not find any significant association, although 32 probes were found significant for at least 1 out of the 5 exposures under study in the cohorts study separately. |
| One-year average ambient $PM_{2.5}$ and $NO_x$ concentrations were predicted using spatiotemporal models | DNA methylation at 2713 expression-associated methylation sites (eMS)[a], in blood samples | Illumina Infinium Human Methylation 450K array | Age, sex, race/ethnicity, household income, education, neighborhood socioeconomic status, smoking status, secondhand smoke, body mass index, recent infection, methyl nutrient intake, physical activity, study site, microarray chip, chip position, | 5 eMS were significantly (FDR <0.05) associated with $PM_{2.5}$, including cg20455854 (*ANKHD1*), cg07855639 (*LGALS2*), cg07598385 (*ANKRD11*), cg17360854 (*BAZ2B*), and cg17360854 (*PPIE*). |

**Table 2** (continued)

| Air pollution exposures | Epigenetic biomarkers,tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| | | | and enrichment scores for neutrophils, B cells, T cells, and natural killer cells | |
| 1-year $PM_{2.5}$ and BC exposures were estimated using LUR model | (Horvath) DNAm-age and specific 353 methylation sites used to calculate DNAm-age[a], in blood samples | Illumina Infinium Human Methylation 450K array | Chronological age and blood cell type, temperature, pack-years, smoking status, season, BMI, alcohol consumption, and education | A 1-$\mu g/m^3$ increase in $PM_{2.5}$ and BC was significantly associated respectively with a 0.52- and 3.02-year increase in DNAm-age. Only $PM_{2.5}$ remained significantly associated with DNAm-age in 2-particle models. Methylation levels from 20 of the 353 CpGs contributing to DNAm-age were significantly associated with $PM_{2.5}$ levels in the 2-particle models. |
| Long term $PM_{2.5}$, $PM_{10}$, BC, and $NO_x$ ambient exposures were estimated via LUR model | (Horvath) DNA methylation age acceleration (DNAmAA), extrinsic epigenetic age acceleration (correlated with immune cell counts, EEAA), and intrinsic epigenetic age acceleration (independent of immune cell counts, IEAA)[a], in blood samples | Illumina Infinium Human Methylation 450K array | Age, sex, physical activity, smoking BMI, systolic blood pressure, diastolic blood pressure, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, hypertension | An interquartile range (0.97 $\mu g/m^3$) increase in $PM_{2.5}$ was associated with a 0.33-year increase in EEAA (CI = 0.01, 0.64; $p = 0.04$). BC and $NO_x$ (indicators or traffic exposure) were associated with DNAmAA and IEAA in women. |
| Short-term ambient BC and particulate sulfate concentrations at monitoring stations for 1 month prior the blood draw | Epigenome-wide scan of the promoter regions of ~19,000 genes[a], in blood samples | 385K RefSeq Promoter Array | Age, blood pressure, smoking status, blood cell composition, and sulfate exposure | Out of 84 MAPK pathway genes considered, 11 and 14 had DNA methylation status highly associated with black carbon exposure and black carbon combined with sulfate. No association was found with the NF-κB pathway. |
| 1-, 2-, 3-, 5-, and 7-day moving average exposures of $PM_{2.5}$ and $PM_{10}$ were calculated from averaged daily ambient concentrations of the pollutants from background monitoring station | Global DNA methylation and hydroxymethylation levels[a], in buccal samples | UPLC-MS/MS | Time, age, sex, average temperature during the respective moving averages, and weekly alcohol consumption | An IQR increase in $PM_{2.5}$ over a 7-day moving average period was significantly associated with a decrease of − 1.47% (− 1.74, − 1.20%) and − 0.043% (− 0.054, − 0.032%) in %5mdC and %5hmdC, respectively. Similarly, for $PM_{10}$, a decrease of − 1.42% (− 1.70, − 1.13%) and − 0.040% (− 0.051, − 0.028%) was observed. |
| Personal $PM_{2.5}$ exposure levels | Global DNA methylation[a], in blood samples | ELISA | Age, BMI, sex, smoking level | A significant multiplicative interaction was detected between rs4344916 on chromosome 2p22.3 and $PM_{2.5}$ exposure on global DNA methylation level ($p = 0.0095$). |
| Ambient $PM_{10}$ mass up to 14 days before the examination and personal $PM_{2.5}$ mass and elemental components on the day of the examination | Global methylation and hydroxymethylation, in blood samples | ELISA | Occupation group, gender, age, BMI, and smoking status | An IQR increase in same-day $PM_{10}$ was associated with increases in 5hmC of 26.1% in office workers ($p = 0.004$), 20.2% in truck drivers ($p = 0.014$), and 21.9% in all participants combined ($p < 0.001$). $PM_{10}$ effects on 5hmC were increasingly stronger when averaged over 4, 7, and 14 days preceding assessment |

**Table 2** (continued)

| Air pollution exposures | Epigenetic biomarkers,tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| | | | | (up to 132.6% for the 14-day average in all participants, $p < 0.001$). $PM_{10}$ effects were also significant after controlling for multiple testing (family-wise error rate; FWER < 0.05). 5hmC was not correlated with personal measures of $PM_{2.5}$ and elemental components (FWER > 0.05). 5mC showed no correlations with $PM_{10}$, $PM_{2.5}$, and elemental components measures (FWER > 0.05). |
| Exposure to air pollutants at the home address was assessed using interpolated $NO_2$, $PM_{10}$, $PM_{2.5}$, and $O_3$ concentrations for various exposure windows (60- to 1-day moving average exposures and yearly averages) | Global DNA methylation[a], in blood samples | HPLC | Gender, age, BMI, physical activity, illness or inflammation in the week before sampling, past smoking habits and passive smoking, cholesterol and vitamin E plasma levels, travel time to work, and average time spent outdoors during a 7-day period | Associations of $NO_2$, PM10, $PM_{2.5}$, and $O_3$ with global methylation were more significant when considering longer-term exposures, especially for $NO_2$. When winter and summer data were analyzed together, $NO_2$, $PM_{10}$, and $PM_{2.5}$ moving average exposures were associated with changes in %5mdC (95% CI) ranging from − 0.04 (− 0.09 to 0.00) to − 0.14 (− 0.28 to 0.00) per IQR increase in pollutant. $NO_2$, $PM_{10}$, $PM_{2.5}$, and $O_3$ moving average exposures were associated with decreased %5mdC (95% CI) varying between − 0.01 (− 0.03 to 0.00) and − 0.17 (− 0.27 to − 0.06) per IQR increase in pollutant in summer but not in winter. |
| 24 h personal $PM_{2.5}$ monitoring 72-h before and during the day of the health visit | DNA methylation at 5 genes (*TNF-α, sICAM-1*, *SCD40L*, and interleukin-6, *TLR2*), in blood samples | Pyrosequencing | Age, sex, BMI, and time-varying temperature, humidity, day of the week, and season, proteins, and methylation measures per experimental plate | Increased $PM_{2.5}$ concentration was negatively associated with DNA methylation at loci for *TNF-α* and *sICAM*-1. |
| 24-h personal $PM_{2.5}$ monitoring 72 h before and during the day of the health visit | *ACE* DNA methylation, in blood samples | Pyrosequencing | Age, sex, body mass index, 3-day average temperature, 3-day average relative humidity, and percentages of neutrophils and lymphocytes | An interquartile range increase (26.78 $\mu g/m^3$) in 24-h average exposure to $PM_{2.5}$ was significantly associated with 1.12 decreases in *ACE* average methylation. |
| Ambient BC and sulfate exposures at monitoring station calculated by averaging over the daily measures in the month prior to a participant's clinic visit | DNA methylation at 27 genes in the asthma pathway, in blood samples | Pyrosequencing | Age | *CCA*, *HLA-DRB5*, *FCER1G*, *IL-5*, and *CCL11* methylation was marginally significantly ($p = 0.05$) associated to both air pollution exposures. Loadings of BC and sulfate show that sulfate, which represents pollution from power plants, is more strongly associated with methylation than black carbon, which measures pollution from traffic. |
| $PM_{2.5}$ concentrations at each participant's residential address up to 1 month prior | DNA methylation at 4 genes *(IFN-γ*, *IL-6*, *ICAM-1*, and *TLR-2*), in blood samples | Pyrosequencing | Age, BMI, race, regular patterns of physical activity, smoking status, pack/years | $PM_{2.5}$ was negatively correlated with *ICAM-1* methylation (betas = − 0.01; CI |

**Table 2** (continued)

| Air pollution exposures | Epigenetic biomarkers,tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| blood draw were estimated using a hybrid LUR and satellite-based mode | | | smoked, alcohol consumption, education level, statin use, batch effects, percentage of lymphocytes, and percentage of neutrophils | 95% = − 0.02, − 0.004), and positively (betas = − 0.01; CI 95% = − 0.02, − 0.004) correlated with *IL-6* methylation (betas = 0.2; CI 95% = 0.03, 0.3) |
| Occupation as active professional FF was considered a proxy for PAH exposure | DNA methylation in 4 genes (GSTP1, IFN- , RAD21, and DUSP22), in blood samples | Methylation specific-PCR and bisulfite sequencing | Age | FF had a higher prevalence of *DUSP22* promoter hypomethylation in blood ($p = 0.03$) and the extent of hypomethylation correlated with duration of firefighting service ($p = 0.04$), but not with age. |
| $PM_{2.5}$ and BC exposures measured at a stationary monitoring site up to 4 weeks before the examination | DNA methylation at *INOS* and *GCR* genes, in blood samples | Pyrosequencing | Age, race, season, time trend, apparent temperature, percent lymphocytes, percent neutrophils, body mass index, diabetes mellitus, and hypertension medication | Increased short- and long-term exposure to both black carbon and $PM_{2.5}$ was associated with decreased *iNOS* methylation. A 1-μg/m$^3$ increase in exposure to black carbon in the 4 h preceding the clinical examination was associated with a 0.9% decrease in 5-methylcytosine (95% CI 0.4, 1.4) in *iNOS*, and a 10-μg/m$^3$ increase in exposure to $PM_{2.5}$ was associated with a 0.6% decrease in 5-methylcytosine (95% CI 0.03, 1.1) in iNOS. Similar results were detected for long-term exposures. No association was found with *GRC* methylation. |
| Long-term $PM_{2.5}$, BC, and $SO_4$ exposures measured at a stationary monitoring site | L1 and *Alu* DNA methylation, in blood samples | Pyrosequencing | Season, apparent temperature, a composite discomfort due to combined heat and high humidity, long-term trends, age, smoking, BMI, prescription medication | An IQR increase in BC over a 90-day period was associated with a decrease of 0.31% 5-methylcytosine (5mC) (95% confidence interval, 0.12–0.50%) in *Alu*. An IQR increase in $SO_4$ over a 90-day period was associated with a decrease of 0.27% 5mC (0.02–0.52%) in L1. |
| Residing in Xuan Wei County a region with high exposure to PAHs due to smoky coal for cooking and heating in homes without chimney | DNA methylation of promoter region of *p16*, *MGMT*, *RASSF1A*, and *DAPK* genes, in sputum samples | Methylation-specific polymerase chain reaction | Gender, age, smoking status, and bronchitis | Promoter methylation of *p16*, *MGMT*, *RASSF1A*, and *DAPK* was detected in 51.4% (55/107), 17.8% (19/107), 29.9% (32/107), and 15.9% (17/107) of the sputum samples, respectively. There were no differences in promoter methylation frequencies of any of these genes according to smoking status or gender of the subjects or between individuals with chronic bronchitis and those without evidence of such a symptom. Only age showed significant effect on *DAPK* methylation status (OR = 1.072; 95% CI = 1.008–1.141, $p = 0.027$). |
| Ambient BC, $PM_{2.5}$, CO, $O_3$, and $NO_2$ concentrations were measured at | DNA methylation at 26 CpG sites located in 9 genes (*CRAT*, *F3*, GCR, *ICAM1*, | Pyrosequencing | Age, height and weight, race, education level, smoking status, cumulative | Associations between IQR increases in 28-day average air pollutant |

**Table 2** (continued)

| Air pollution exposures | Epigenetic biomarkers,tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| monitoring stations 4 h to 28 days prior lung function test execution | *IFN-γ*, *IL-6*, *iNOS*, *OGG1*, *TLR2*), in blood samples | | smoking in pack-years, season of the medical examination, day of the week, visit number, temperature and relative humidity, physician-diagnosed chronic lung condition | concentrations and the lung function measures were significantly higher in participants with low (< median) methylation in *TLR2* at position 2 and position 5 and high (≥ median) methylation in *GCR*. |
| Personal exposure to 8 elemental components of $PM_{2.5}$ (Al, Si, S, K, Ca, Ti, Fe, and Zn) were measured during 8 working hours | DNA methylation on tandem repeats (3 CpG sites from *SATa*, 4 CpG sites from *NBL2*, and 4 CpG sites from *D4Z4*), in blood samples | Pyrosequencing | Occupation, $PM_{2.5}$, measure day, age, sex, BMI, number of cigarettes smoked during examination time, and usage of central heating | *NBL2* methylation was positively associated with concentrations of Si (0.121, 95% CI 0.030, 0.212; FDR = 0.047) and Ca (0.065, 95% CI 0.014, 0.115; FDR = 0.047) in truck drivers. In office workers, *SATa* methylation was positively associated with concentrations of S (0.115, 95% CI 0.034, 0.196; FDR = 0.042). |
| Ambient $PM_{10}$ mass up to 14 days before the examination and personal $PM_{2.5}$ mass and elemental components on the day of the examination | DNA methylation on tandem repeats (3 CpG sites from *SATa*, 4 CpG sites from *NBL2*, and 4 CpG sites from *D4Z4*), in blood samples | Pyrosequencing | Age, sex, BMI, smoking status, number of cigarettes per day, work hours per day, outdoor humidity, and temperature | Overall, an interquartile increase in personal $PM_{2.5}$ and ambient $PM_{10}$ levels was associated with a significant covariate-adjusted decrease in *SATa* methylation (21.35%, $p = 0.01$; and 21.33%, $p = 0.01$, respectively). These effects were more pronounced and statistically significant in truck drivers (22.34%, $p = 0.02$; 21.44%, $p = 0.06$ vs. office workers 20.95%, $p = 0.26$; 21.25%, $p = 0.12$, respectively). In all participants combined, *NBL2* and *D4Z4* showed no associations with the levels of personal $PM_{2.5}$, personal EC, or ambient $PM_{10}$. However, ambient $PM_{10}$ was negatively correlated with *NBL2* methylation in truck drivers (21.38%, $p = 0.03$) but not in office workers (1.04%, $p = 0.13$). |
| Short-term exposure to $PM_{2.5}$ and its constituents were measured at a fixed-site monitor | DNA methylation at 3 CpG sites located in *NOS2A*, in buccal samples | Pyrosequencing | Age, gender, body mass index, education, mean temperature, relative humidity, week, day of the week, duration of COPD and chronic comorbidities | An IQR increase in total $PM_{2.5}$ was associated with decreases methylation of *NOS2A* of 1.19, 1.63, 1.62, 1.17, and 1.14, respectively, with $PM_{2.5}$, organic carbon, element carbon, $NO_3^-$, and $NH_4^+$. |
| Daily mean of $PM_{10}$ concentrations were measured at monitoring station and assigned at participants' residence at 1 to 14 days back prior the enrolment | DNA methylation of 3 genes (*CD14*, *TLR4* and *TNF-α*), in peripheral blood mononuclear cells | Pyrosequencing | BMI, age, sex, percentage of neutrophils, smoking habits, position, run, batch, interaction terms among position, run and batch | $PM_{10}$ effect on *CD14* methylation was observed in days 4–5–6 (β4days = − 0.12, pP4days = 0.029; β5days = − 0.12, P5days = 0.028; β6days = − 0.155, P8days = 0.006), and on *TLR4* methylation in days 6–7–8 (β6days = − 0.169, P6days = 0.048; β7days = − 0.260, P7days = 0.006; β8days = − 0.187, P8days = 0.059). |

**Table 2** (continued)

| Air pollution exposures | Epigenetic biomarkers,tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|
| Long-term total suspended particulate (TSP) exposure was generated via inverse distance squared weighed interpolation, and traffic exposure (TE) was estimated through a region-specific traffic dispersion model | DNA methylation of 9 genes (*SFN*, *SCGB3A1*, *RARB*, *GSTP1*, *CDKN2A*, *CCND2*, *BRCA1*, *FHIT*, and *SYK*), in breast tumor | Pyrosequencing | Age, estrogen receptor status, current smoking status, secondhand smoke exposure before age 20 | TSP at the time of the woman's first birth was associated with:– Decreased methylation of *SCGB3A1* (OR = 0.48, 95% CI 0.23–0.99);– Increased methylation of *SYK* (OR = 1.86, 95% CI 1.03–3.35).TE at menarche was associated with increased methylation of *SYK* (OR = 2.37, 95% CI 1.05–5.33).TE exposure was associated with decreased methylation of *CCND2* at first birth (OR = 0.40, 95% CI 0.17–0.93), 20 years prior to diagnosis (OR = 0.48, 95% CI 0.26–0.89), and 10 years prior to diagnosis (OR = 0.55, 95% CI 0.30–1.00). |
| 4-week moving averages of $PM_{2.5}$ and BC exposures calculated from hourly measured concentration at monitoring stations | DNA methylation at 5 genes (*F3*, *ICAM-1*, IFN-γ, *TRL-2*, and *IL-6*), in blood samples | Pyrosequencing | Temperature, relative humidity, day of the season, batch of methylation measurement, age, diabetes, body mass index, smoking status, statin use, as well as percentages of neutrophils and lymphocytes in differential blood count | Black carbon and $PM_{2.5}$ concentrations were associated with significantly lower methylation in the lower tails of the *IFN-γ* and *ICAM*-1 methylation distributions. In particular, a 3.4-μg/m$^3$ increase in $PM_{2.5}$ mass concentration was associated with a 0.18% 5mC decrease on the 20th percentile of *ICAM-1* methylation. |
| Ambient particle number, BC, sulfates, and ozone exposures were measured at monitoring stations up to 4 weeks prior the blood draw | DNA methylation at 5 genes (*F3*, *ICAM-1*, *IFN-γ*, *TRL*-2, and *IL-6*), in blood samples | Pyrosequencing | Temperature, relative humidity, seasonal sine and cosine, season, batch of methylation measurement, age, diabetes, body mass index, smoking status, statin use, as well as percentages of neutrophils and lymphocytes in differential blood count | Increase in air pollutant concentrations was significantly associated with *F3*, *ICAM-1*, and *TLR-2* hypomethylation, and *IFN-γ* and *IL-6* hypermethylation. An interquartile range increase in black carbon concentration averaged over the 4 weeks prior to assessment was associated with a 12% reduction in F3 methylation (95% CI − 17 to − 6%). For some genes, the change in methylation was observed only at specific locations within the promoter region. |
| Short-term $PM_{2.5}$, BC, and $SO_4$ exposures were measured at a stationary monitoring site | L1 and *Alu* DNA methylation, in blood samples | Pyrosequencing | Age, body mass index, cigarette smoking, pack-years, statin use, fasting blood glucose, diabetes mellitus, percent lymphocytes and neutrophils in differential blood count, day of the week, season, and outdoor temperature | L1 methylation decreased after recent exposure to higher black carbon ($b$ − 0.11; 95% confidence interval [CI], − 0.18 to − 0.04; $p$ = 0.002) and $PM_{2.5}$ ($b$ − 0.13; 95% CI, − 0.19 to − 0.06; $p$ = 0.001 for the 7-day moving average). In 2-pollutant models, only black carbon, a tracer of traffic particles, was significantly associated with L1 methylation ($b$ = − 0.09; 95% CI, − 0.17 to − 0.01; $p$ = 0.03). No association was found with *Alu* methylation. |

*BTDAS*: Beijing Truck Driver Air Pollution Study; *DMR*: differentially methylated region; EPIC: European Prospective Investigation into Cancer and Nutrition; *KORA*: Cooperative Health Research in the Augsburg Region; *L1*: LINE-1; *LUR*: land use regression; *NAS*: Normative Aging Study; *MESA*: Multi-Ethnic Study of Atherosclerosis; *WEB*: Western New York Exposures and Breast Cancer

[a] Untargeted exploration of the methylome

**Table 3** The characteristics of the seven experimental studies included in the present systematic review investigating DNA methylation and air pollution. Articles result from a search carried out in April 2018 using a combination of MeSH terms and keywords in two different search engines (PubMed and Scopus) and have been selected using specific inclusion criteria derived from the PECO statement. For each study, the table reports the first author and the year of publication along with the reference, the relevant country, the study design, the study period, the population size, age and cohort from which data were derived, the air pollution exposures examined, the epigenetic biomarkers analyzed along with the method of detection, the confounders identified, the main findings, and the risk estimates. Studies using untargeted exploration of the methylome are presented first followed by those using targeted approaches

| No. | Authors, year | Country | Study design | Study period | Population, age | Study name from which data are derived | Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Li et al., 2018 | China | Randomized, double-blind crossover trial | 2015 | 36 healthy, nonsmoking college students, mean age 19.8 years | | $PM_{2.5}$ in 10 dormitory rooms was modified using true or sham air purifiers for 48 h with a 2-week washout interval | Epigenome-wide DNA methylation[a], in blood samples | Illumina Infinium Human Methylation 850K array | Age, sex, BMI, and leukocyte compositions | 49 CpG loci were differentially methylated (FDR < 0.01) in the high- and low-exposure scenarios of $PM_{2.5}$. These CpGs were annotated to 31 genes implicated in insulin resistance, glucose and lipid metabolism, inflammation, oxidative stress, platelet activation, and cell survival and apoptosis. |
| 2 | Zhong et al., 2017 [80] | Canada | Placebo-controlled crossover pilot intervention trial | 2013–2014 | 10 healthy volunteers, age range 18-60 years | | Participants were exposed to (i) placebo for 2 weeks, (ii) followed by the baseline sham experiment (2 h, particle-free medical air, exposure one), (iii) placebo for 4 weeks, (iv) and then were exposed to $PM_{2.5}$ (2 h, target concentration: 250 μg/$m^3$, exposure 2), (v) vitamin B supplementation for 4 weeks after exposure 2, and (vi) then were exposed again to $PM_{2.5}$ (2 h, target concentration 250 μg/$m^3$, exposure 3) | Epigenome-wide DNA methylation[a], in blood samples | Illumina Infinium Human Methylation 450K array | Season, chamber humidity, and temperature | Top CpG loci associated with $PM_{2.5}$ (based on significance and size of association) related to a known gene were cg06194186 on the *CPO* gene (coef = 1.74, *p* value = 2.3e-5) and cg17157498 on *NDUFS7* gene (coef = − 1.67, *p* value = 1.3e-5). |
| 3 | Clifford et al., 2017 [81] | Canada | Randomized crossover--controlled exposure study | | 17 volunteers, age range 20-46 years | | Participants were exposed to 2 conditions: diesel exhaust (300 mg $PM_{2.5}$/$m^3$) and allergen or filtered air and allergen | Epigenome-wide DNA methylation[a], in blood samples | Illumina Infinium Human Methylation 450K array | | Exposure to allergen alone, diesel exhaust alone, or allergen and diesel exhaust together (coexposure) led to significant changes in 7 CpG sites at 48 h. However, when the same lung was exposed to allergen and diesel exhaust but separated by approximately 4 weeks, significant changes in more than 500 sites were observed. Furthermore, sites of differential methylation differed depending on which exposure was experienced first. |
| 4 | Jiang et al., 2014 [82] | Canada | Double-blind crossover study | | 16 adults affected from asthma, age range 19-35 years | | Each subject was subjected to either filtered air (FA) or diesel exhaust (nominally, 300 μg/$m^3$ $PM_{2.5}$) for 2 h on 2 separate occasions at least 2 weeks apart | Epigenome-wide DNA methylation[a] and probes overlapping with *Alu*, L1 repetitive elements and microRNAs | Illumina Infinium Human Methylation 450K array | | At epigenome-wide level, 2827 CpG sites were affected by exposure to diesel exhaust but not filtered air, and these sites enriched for genes involved in protein kinase and NF-kB |

**Table 3** (continued)

| No. | Authors, year | Country | Study design | Study period | Population, age | Study name from which data are derived | Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | miR-21, miR-30e, miR-215, and miR-144, in blood samples | | | pathways. Analysis of pre-/post exposure to diesel exhaust revealed significant changes in CpGs that became less methylated, with a site residing within *GSTP1* being among the significant hits. Diesel exhaust-associated change was also found for CpG sites overlapping with *Alu* and L1 elements and a site within miR-2. |
| 5 | Tobaldini et al., 2018 [83] | Italy | Double-blind, randomized, crossover, controlled study | | 12 young volunteer healthy males, mean age 25.1 years | | Subject inhaled: (i) filtered air mixture and (ii) filtered air plus particulate mixture ($PM_{10}$, $PM_{2.5}$, $PM_{1.0}$, and $PM_{0.5}$) | DNA methylation at L1 and *Alu* and at candidate genes (*SATalpha*, *IL-6*, *ICAM*, *TLR2*, *TLR4*, *ET1*, *IFN-γ*, *iNOS*, *eNO*), in blood samples | Pyrosequencing | | Using all measures combined (mixed models using T1, T2, and T3), fine and ultrafine PM fractions showed significant associations with *IFN-γ* methylation. In particular, particulate matter fraction with aerodynamic diameter comprised between 0.3 and 0.5 μm was significantly associated to *IFN-γ* methylation (β90th–10th = 4.40, $p$ value < 0.0001) such as PM fraction with aerodynamic diameter comprised between 0.5 and 1 μm (β90th–10th = 6.53, $p$ value < 0.0001). Larger fractions were not significantly associated to *IFN-γ* methylation (PM 1–3 μm: β90th–10th = 2.0, $p$ value = 0.1790; PM 3–5 μm: β90th–10th = 0.04, $p$ value = 0.97; PM 5–10 μm: β90th–10th = − 0.13, $p$ value = 0.9369; PM > 10 μm: β90th–10th = − 1.28, $p$ value = 0.3886). No other significant association was found for the other methylation sites under investigation. |
| 6 | Chen et al., 2016 [84] | China | Randomized, double-blind crossover trial | 2014 | 35 healthy college students, mean age 23 years | | $PM_{2.5}$ in 10 dormitory rooms through using true or sham air purifiers for 48 h with a 2-week washout interval | DNA methylation at L1 and *Alu* repetitive elements and in 10 candidate genes, in blood samples | Pyrosequencing | Percentages of neutrophils and lymphocytes in differential blood count, indoor temperature, and indoor relative humidity | An IQR increase (64 μg/m$^3$) in $PM_{2.5}$ was significantly associated with reduction of methylation in L1 (1.44%), 1 proinflammatory gene (*CD40LG*, 9.13%), 2 procoagulant genes (*F3*, 15.20%; *SERPINE1*, 3.69%), and 2 provasoconstriction genes (*ACE*, 4.64%; EDN1, 9.74%). |

**Table 3** (continued)

| No. | Authors, year | Country | Study design | Study period | Population, age | Study name from which data are derived | Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | Bellavia et al., 2013 [85] | USA | Double-blind randomized placebo-controlled crossover study | 2008–2010 | 15 healthy volunteers, mean age 27.7 years | | Subjects were exposed to: (i) fine concentrated ambient particles (CAPs), (ii) coarse CAPs, and (iii) high efficiency-particulate-air-filtered medical air | *Alu* and L1 DNA methylation and DNA methylation at 4 genes (*TLR4*, *IL-6*, *IL-12*, and *iNOS*), in blood samples | Pyrosequencing | | Compared to control fine CAPs exposure lowered *Alu* methylation (betas = 0.74, adjusted $p = 0.03$); coarse CAPs exposure lowered *TLR4* methylation (betas = 0.27, adjusted $p = 0.04$). No significant differences were observed in any exposures for L1, *IL-6*, *IL-12*, or *iNOS* methylation. |

*L1*: LINE-1

[a] Untargeted exploration of the methylome

to observations in the cohort studies, one experimental study found a negative association between PM and L1 methylation levels (no. 6), though another did not find any significant effect (no. 5). Exposure to fine concentrated ambient particles lowered *Alu* methylation but did not seem to have an effect on L1 (no. 7). Though the experimental studies conducted so far using an EWAS approach (nos. 1–4) are based on few subjects ($n \leq 36$), they have identified several CpGs that are significantly altered by $PM_{2.5}$ exposure (nos. 1 and 2) and a large number of CpGs that are differentially methylated in response to diesel exhaust (500 and 2827 CpGs in nos. 3 and 4, respectively).

## Noncoding RNA (MiRNA and LncRNA)

Only 15 studies investigated the effect of air pollution on noncoding RNAs (ncRNAs) (Table 4), representing only 18% of the literature mined in this work (Fig. 2b). All these studies analyzed miRNAs except one that focused on lncRNAs (no. 15). Five studies had an experimental design (crossover; nos. 1, 6, and 8–10), while the remaining ten were observational studies (cohort and cross-sectional). Most of the studies investigated the effects of short-term exposure to air pollutants, while fewer focused on chronic exposures including the in utero time window (nos. 7, 11, and 15). All the studies examined adults, apart from two whose participants were newborns (no. 11) or school children (no. 13). Noncoding RNA was extracted from blood samples in ten reports, while lung tissue, bronchial brushing, sputum, saliva, and placenta samples were used in the remaining studies. Given the relatively small and diverse sample of publications available on ncRNAs and air pollution, it is difficult to currently draw generalizable conclusions. However, compared to DNA methylation, the ncRNA studies with observational and untargeted RNA approaches (RNA-seq or large panel of profiled ncRNAs) have been able to detect several significantly regulated ncRNAs in response to various air pollutants, even though their sample sizes were overall smaller (ranging between 22 and 1630 samples; median = 90; Table 3) relative to those reported with similar study designs on DNA methylation (ranging between 141 and 2956 samples, median = 839; Table 2). *miR-21* was frequently reported to be associated with air pollution in different studies (Table 4).

## Chromatin Regulation

Only two studies on chromatin modification related to environmental exposures were identified (Table 4). Both studies were carried out in China and used an observational design to investigate the association of personal (no. 17) and environmental PM (nos. 16 and 17) exposures on histone modifications in adult blood. One study characterized genome-wide profiles of histone H3 lysine 27 acetylation (H3K27ac) using chromatin immunoprecipitation sequencing but based on a small sample size ($n = 4$),

**Table 4** The characteristics of the 17 studies included in the present systematic review investigating noncoding RNAs (15 studies) and chromatin remodeling (two studies) and air pollution. Among the 15 studies dealing with noncoding RNAs, 14 investigated miRNAs and the remaining one lncRNAs. Articles result from a search carried out in April 2018 using a combination of MeSH term and keywords in two different search engines (PubMed and Scopus) and have been selected using specific inclusion criteria derived from the PECO statement. For each study, the table reports the first author and the year of publication along with the reference, the relevant country, the study design, the study period, the population size, age and cohort from which data were derived, the air pollution exposures examined, the epigenetic biomarkers analyzed along with the method of detection, the confounders identified, the main findings, and the risk estimates. Studies using untargeted explorations are presented first followed by those using targeted approaches

| No. | Authors, year | Country | Study design | Study period | Population, age | Study name from which data are derived | Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| miRNA | | | | | | | | | | | |
| 1 | Krauskopf et al., 2018 [86••] | UK | Randomized experimental crossover study | | 24 adults (healthy and diseased), mean age 65.1 years | Oxford street | Personal ambient air pollution levels of $PM_{2.5}$, $PM_{10}$, UFP, BC, and $NO_2$ while subjects were walking for 2 h in Oxford street and, after 3 weeks, for other 2 h in traffic-free Hyde Park | 595 cmiRNAs[a], in blood samples | RNA-seq | Sex, age, BMI, and disease state (healthy, IHD, or COPD) | In total, 54 cmiRNAs were associated with $NO_2$ (6), UFP (7), $PM_{2.5}$ (23), BC (26), and $PM_{10}$ (28) exposures. Bioinformatics analysis suggests that these cmiRNAs reflect the adverse consequences of traffic pollution-induced toxicity in target tissues including lung, heart, kidney, and brain. |
| 2 | Liu et al., 2018 [87] | China | (Pilot) cohort study | 2014–2015 | 26 patients (9 affected by COPD in the Beijing group, 8 affected by COPD in the migratory group, and from 9 healthy control subjects), age range 51–82 years | | Residence in polluted Beijing vs migration during the winter period in Hainan (characterized by better air quality) were used as proxy for air pollution exposure | 1539 miRNAs[a], in blood samples | RNA-seq | | 9 miRNAs (hsa-miR-142-5p, hsa-miR-16-2-3p, hsa-miR-186-5p, hsa-miR-1180-3p, hsa-miR-148b-3p, hsa-miR-7976, hsa-miR-144-3p, hsa-miR-223-5p, and hsa-miR-7706) were differentially expressed between resident and migratory COPD groups. |
| 3 | Pergoli et al., 2017 [88] | Italy | Cross-sectional study | 2010–2015 | 1630 obese adults (883 and 747 involved in discovery and validation stages, respectively), mean age 52.4 years | SPHERE | Short-term exposure to $PM_{10}$ (1-week lag exposure time window before the day of recruitment) calculated from daily $PM_{10}$ concentration estimates at participants' residence | 545 extracellular vesicles miRNAs[a], in blood samples | miRNA RT-qPCR | Age, sex, BMI, smoking status, and apparent temperature | 46 miRNAs were significantly associated with the 1-week lag–$PM_{10}$ exposure. 9 (let-7c-5p; miR-106a-5p; miR-143-3p; miR-185-5p; miR-218-5p; miR-331-3p; miR-6425p; miR-652-3p; miR-99b-5p) out of the 46 miRNAs survived at validation and were involved in CVD network according functional analysis |
| 4 | Hou et al., 2016 [89]. | China | Cross-sectional study | 2008 | 60 truck drivers and 60 office workers, mean age, respectively 33.5 and 30.3 years | BTDAS | Ambient $PM_{10}$, personal $PM2_{.5}$, and personal EC measurements on the study day | 654 endogenous human--associated miRNAs and 80 viral miRNAs[a],in blood samples | Multiplex miRNA profiling | Age, sex, BMI, smoking status, number of cigarettes smoked on the examination day, examination day, work hours per week, outdoor temperature, and dew point | Short-term EC average level was associated with differential expression of 46 human miRNAs and all 7 viral miRNAs at FDR significance level < 20%. MiRNA profiles differed in the two groups: 3 viral miRNAs (EBV-miR-BART2-5p, EBV-miR-BART6-5p, and KSHV-miR-K12–9) were significant in office workers and 4 |

**Table 4** (continued)

| No. | Authors, year | Country | Study design | Study period | Population, age | Study name from which data are derived | Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | viral miRNAs (EBV-miR-BHRF-1-3, EBV-miR-BHRF-1-1, EBV-BART-9, and HIV1-miR-H1) in truck drivers. Gene function and pathway analyses demonstrated that the putative mRNAs targeted by the differentially expressed miRNAs are implicated in inflammation in office workers, and in cellular proliferation- and differentiation-related pathways in truck drivers. No significant miRNA was associated with $PM_{2.5}$ or $PM_{10}$. |
| 5 | Motta et al., 2016 [90] | Italy | Cross-sectional study | 2010–2011 | 90 obese adults, mean age 51.6 years | SPHERE | Short-term exposure to $PM_{10}$ (48 h lag exposure time window before the day of recruitment) calculated from daily mean of $PM_{10}$ concentration at participants' residence | 377 human miRNAs[a], in blood samples | miRNA RT-qPCR | Age, sex, BMI, smoking habits, percentage of granulocytes, recruitment date, and ambient apparent temperature | 9 miRNAs (miR-26a, miR-101, miR-145, miR-197, miR-30b, miR-345, miR-425–5p, miR-331, and miR-140–3p) were negatively associated with $PM_{10}$ lag exposure levels 48 h before the recruitment day at a FDR threshold < 0.1. miR-101 mediated the effects of particle exposure on diastolic BP, possibly influencing the risk of developing CVD. |
| 6 | Rider et al., 2016 [91] | Canada | Double-blinded crossover exposure study | | 15 nonsmokers healthy volunteers with atopy (skin prick positive) to house dust mite, age range 19–49 years | | Participants were exposed separately for 2 h to DE (standardized to 300 mg $PM_{2.5}/m^3$) and filtered air (negative control for DE), followed by saline-controlled segmental bronchial allergen challenge | 800 miRNAs[a], in bronchial brushings | Multiplex miRNA profiling | | Robust linear regression showed that DE plus saline modulated the highest number of miRNAs relative to control (filtered air plus saline). In the mixed model analysis of DE, 6 miRNAs had $p$ values of 0.05 or less (miR-22-3p, 197-3p, 135b-5p, 15b-5p, 141-3p, and 92a-3p), but all had $q$ values of more than 0.2. |
| 7 | Rodosthenis et al., 2016 [92] | USA | Cohort study | 2000–2008 | 22 elderly man, mean age 75 years | NAS | $PM_{2.5}$ exposures measured at a stationary monitoring site up to 1 year before the examination | 800 extracellular vesicles miRNAs[a], in blood samples | Multiplex miRNA profiling | Age, BMI, pack-years of smoking, total miRNA counts, and counts of RBCs, WBCs, and platelets | 16 miRNAs were found statistically significantly associated with either the 6-month or 1-year $PM_{2.5}$ moving averages. In silico pathway analysis on $PM_{2.5}$-associated extracellular vesicles miRNAs identified several key CVD-related pathways including oxidative |

**Table 4** (continued)

| No. | Authors, year | Country | Study design | Study period | Population, age | Study name from which data are derived | Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | stress, inflammation, and atherosclerosis. |
| 8 | Fry et al., 2014 [93] | USA | Experimental crossover study | | 20 healthy nonasthmatic adult subjects, age range 18–37 years | | Subject were exposed to 0.4 ppm $O_3$ for 2 h | 534 human miRNAs[a], in sputum samples | miRNA array | Age, atopy, body mass index, sex, and race | $O_3$ increased the expression levels of 10 miRNAs, namely miR-132, miR-143, miR-145, miR-199a, miR-199b-5p, miR-222, miR-223, miR-25, miR-424, and miR-582-5p, associated with a wide range of biological functions and disease signatures, noted among them inflammation and immune-related disease. |
| 9 | Yamamoto et al., 2013 [94] | Canada | Randomized crossover, double-blind experiment | | 13 volunteers, age range 19–35 years | | Subjects were exposed on 3 different days to each of 3 conditions: filtered air exposure plus placebo (FAP), DE exposure (300 μg $PM_{2.5}/m^3$) plus placebo (DEP), and DE exposure plus *N*-acetylcysteine (NAC) supplementation (DEN) | 734 human and human--associated viral miRNAs[a], in blood samples | Multiplex miRNA profiling and miRNA RT-PCR | | Expression of miR-21, miR-30e, miR-215, and miR-144 was significantly associated with DEP, and miR-144 was further validated by RT-qPCR. miR-144 and miR-21 significantly decreased after *N*-acetylcysteine supplementation. |
| 10 | Chen et al., 2018 [95] | China | Double-blind, randomized crossover study | 2015 | 55 volunteer students, mean age 20 years | | Participants used in the center of their rooms: (i) a true air purifier for 9 days; (ii) a sham air purifier for other 9 days after 12 days of washout | 10 human miRNA associated to cytokines, in blood samples | miRNA RT-qPCR | Age, gender, BMI, and daily average temperature and relative humidity | IQR increases in $PM_{2.5}$ exposures (time-weighted) were significantly associated with lower estimated mean expression of 5 of the 10 miRNAs under study (miR-21-5p, miR-187-3p, miR-146a-5p, miR-1-3p, and miR-199a-5p) predicted to target mRNAs of *IL-1*, *TNF*, *TLR2*, and *EDN1*. |
| 11 | Tsamou et al., 2017 [96] | Belgium | Cohort study | 2010–2014 | 210 mother–newborn pairs,mean gestational age 39.2 weeks | ENVIRONAGE | Pregnancy $PM_{2.5}$ and $NO_2$ exposures were estimated using a spatial temporal interpolation method (kriging) | miR-16, miR-20a, miR-21, miR34a miR-146a, and miR-222, in placenta samples | miRNA RT-qPCR | Maternal age, pregestational BMI, smoking status, educational status, parity, and newborn's gender, gestational age, ethnicity, seasonality, and apparent temperature | $PM_{2.5}$ exposure during the second trimester of pregnancy was negatively associated with miR-21 (− 33.7%, 95% CI − 53.2 to − 6.2, $p = 0.022$), miR-146a (− 30.9%, 95% CI − 48.0 to − 8.1, $p = 0.012$), and miR222 (− 25.4%, 95% CI − 43.0 to − 2.4, $p = 0.034$), while expression of miR-20a and miR-21 was positively associated with first trimester exposure. No association was found for $NO_2$ exposure. |
| 12 | Louwies et al., 2016 [97] | Belgium | Cross-sectional study | 2014–2015 | 50 participants, mean age 32 years | | Ambient $PM_{10}$ measured at monitoring stations up to 1 week before the visits | miR-21, miR-146a and, miR-222, in blood samples | miRNA RT-qPCR | Age, gender, BMI, blood pressure, location, day of the week, alcohol and caffeine | Short-term increase of 10 $mg/m^3$ $PM_{10}$ during the 24 h preceding the study visit was associated with a 6.6% decrease (95% CI 11.07, |

**Table 4** (continued)

| No. | Authors, year | Country | Study design | Study period | Population, age | Study name from which data are derived | Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | consumption, and outdoor temperature | 2.17; $p = 0.0038$) in miR-21 expression and a 6.7% decrease (95% CI 10.70, 2.75; $p = 0.0012$) in miR-222 expression. |
| 13 | Vriens et al., 2016 [98] | Belgium | Cross-sectional study | 2011–2013 | 80 children, mean age 10.4 years | COGNAC | Ambient $PM_{2.5}$ and UFP measured at school on the day of the visit and $PM_{2.5}$ exposure measured at the residential address up to 24 h before the visit | miR-222 and miR-146a, in saliva | miRNA RT-qPCR | Sex, age, BMI, exposure to passive smoking, maternal education level, time and day of examination, time/week spent watching TV, and the extracellular RNA concentrations | 29.9% increment (95% CI 10.6–49.1%) in miR-222 expression ($p = 0.0027$) was associated with an IQR increment in UFP concentration, while no association was found for $PM_{2.5}$ levels. No associations were found between both air pollution exposures and miR-146a. |
| 14 | Fossati et al., 2014 [99] | USA | Cohort study | 1963 onward | 153 elderly man, mean age 77.4 years | NAS | $PM_{2.5}$, BC, $SO_4$, and organic carbon exposures measured at a stationary monitoring site up to 4 weeks before the examination | 14 miRNAs, in blood samples | miRNA RT-PCR | Age, BMI, cigarette smoking, pack-years of smoking, granulocytes, date, seasonality, and miRNA batch | An overall association in the negative direction was found for pollutants in all the exposure windows and 8 miRNAs (miR-1, miR-126, miR-135a, miR-146a, miR-155, miR-21, miR-222, and miR-9). |
| lncRNA | | | | | | | | | | | |
| 15 | Wei et al., 2016 [100] | China | Cross-sectional study | | 148 patients with nonsmall cell lung cancers divided by area of residence: 62 from highly polluted region (HPR) and 86 from control regions (CR), age 73% (> 65 years), 23% (<= 65 years) | | Residence in HPR because of coal burning industrial plants vs. residence in CR | lncRNAs[a], in normal lung tissue and nonsmall cell lung cancers | lncRNA microarray | | HPR residing patients had more dysregulated lncRNAs (379) then patients from control regions (300). The lncRNA CAR intergenic 10 (CAR10) was upregulated in 62.9% of HPR-resident patients, which was much higher than in patients from control regions (37.2%; $p = 0.002$). |
| Chromatin | | | | | | | | | | | |
| 16 | Liu et al., 2015 [101] | China | Cross-sectional study | | 4 healthy subjects | | Participants were assigned to low- or high-exposure groups according to measurements of ambient $PM_{2.5}$ levels | Genome-wide H3K27ac modifications[a], in blood samples | ChIP sequencing | | H3K27ac peaks that aggregated on TSS and enhancer regions were higher in the individuals with high $PM_{2.5}$ exposure compared to low-exposed individuals. Gene set enrichment showed differential H3K27ac loci were most significantly enriched in pathways related to immune response. |
| 17 | Zheng et al., 2017 [102] | China | Cross-sectional study | 2008 | 60 truck drivers and 60 indoor office workers, mean age 33.5 and 30.3 years, respectively | BTDAS | Ambient $PM_{10}$ exposure on the visit day and 14 days before and personal $PM_{2.5}$ and BC exposures on the visit day and 1–2 weeks apart | Histone 3 modifications (total histone H3, H3K9ac, H3K9me3, H3K27me3, and | ELISA | Occupation group, sex, age, BMI, smoking status, and day of the week | In all participants. each microgram per cubic meter increase in 14-day average ambient $PM_{10}$ exposure was associated with lower H3K27me3 ($\beta = -1.1\%$, 95% CI −1.6, −0.6); occupation-stratified analyses showed associations |

**Table 4** (continued)

| No. | Authors, year | Country | Study design | Study period | Population, age | Study name from which data are derived | Air pollution exposures | Epigenetic biomarkers, tissue | Detection technique | Confounders | Main findings and risk estimates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | H3K36me3)[a], in blood samples | | | between BC and both H3K9ac and H3K36me3 were stronger in office workers ($\beta = 4.6\%$, 95% CI 0.9, 8.4; and $\beta = 4.1\%$, 95% CI 1.3, 7.0, respectively) than in truck drivers ($\beta = 0.1\%$, 95% CI − 1.3, 1.5; and $\beta = 0.9\%$, 95% CI − 0.9, 2.7, respectively; both $p$ interaction < 0.05); no associations were found for personal $PM_{2.5}$ or elemental components. |

*BTDAS*: Beijing Truck Driver Air Pollution Study; *COGNAC*: COGNition and Air pollution in Children; *ENVIRONAGE*: Environmental Influences on Early Ageing Study; *NAS*: Normative Aging Study

[a] Untargeted exploration of the epigenetic marker

while the other study measured candidate global histone H3 modifications (H3K9ac, H3K9me3, H3K27me3, and H3K36me3, with "me3" indicating trimethylation) via ELISA. Both studies showed that PM levels positively associate with a decondensed (i.e., active) chromatin structure around gene promoters, evidenced by increased H3K27ac (no. 16) or decreased H3K27me3 (no. 17) levels in those regions. Moreover, the $PM_{2.5}$-associated differential H3K27ac markers were enriched in genes involved in immune cell activation (no. 16).

## Discussion

We conducted a systematic review of the link between epigenetics and constituents of air pollution in humans. We identified 82 eligible manuscripts, among which 65 analyzed DNA methylation, 15 ncRNAs, and 2 chromatin modifications. In general, several studies profiling epigenome-wide associations as well as hypothesis-driven analyses show evidence that air pollution differentially affects epigenetic parameters at the genes that belong to various pathways including inflammation and immune system, DNA damage response, and cardiovascular and neurological functions. Because of their heritable but potentially reversible properties, epigenetic mechanisms have emerged as a promising biological explanation linking events and exposures across life to long-term health. Epigenetic alterations of stem cells in particular are believed to play a pivotal role in cell programming and may explain why exposures in early life can have effects that can be detectable later in life [105]. Since epigenetic changes can be mitotically heritable, distinct methylation patterns associated with air pollution could be considered as a memory of previous exposures that persist for decades [106]. One third of the studies identified in this work were performed in children or in newborns (Fig. 2a), with significant findings suggesting possible mechanisms underlying the developmental origin of health and disease (DOHaD) theory. Several novel CpG sites and mechanisms, which may create a molecular basis for the association between air pollution and health outcomes, have been detected (Tables 1, 2, 3, and 4), but more studies are needed to establish causality.

### DNA Methylation

DNA methylation was the most widely investigated epigenetic mechanism among the screened studies. Though EWASs have proven to be powerful in identifying and replicating extensive exposure-associated epigenetic alterations, such as maternal tobacco smoke [107], results for particulate air pollution are still sparse and the magnitude of differential methylation detected is generally much lower compared to results found for smoking, which is also an airborne exposure. One reason likely lies in the fact that, apart from being a different type of exposure, air pollution levels are more prone to

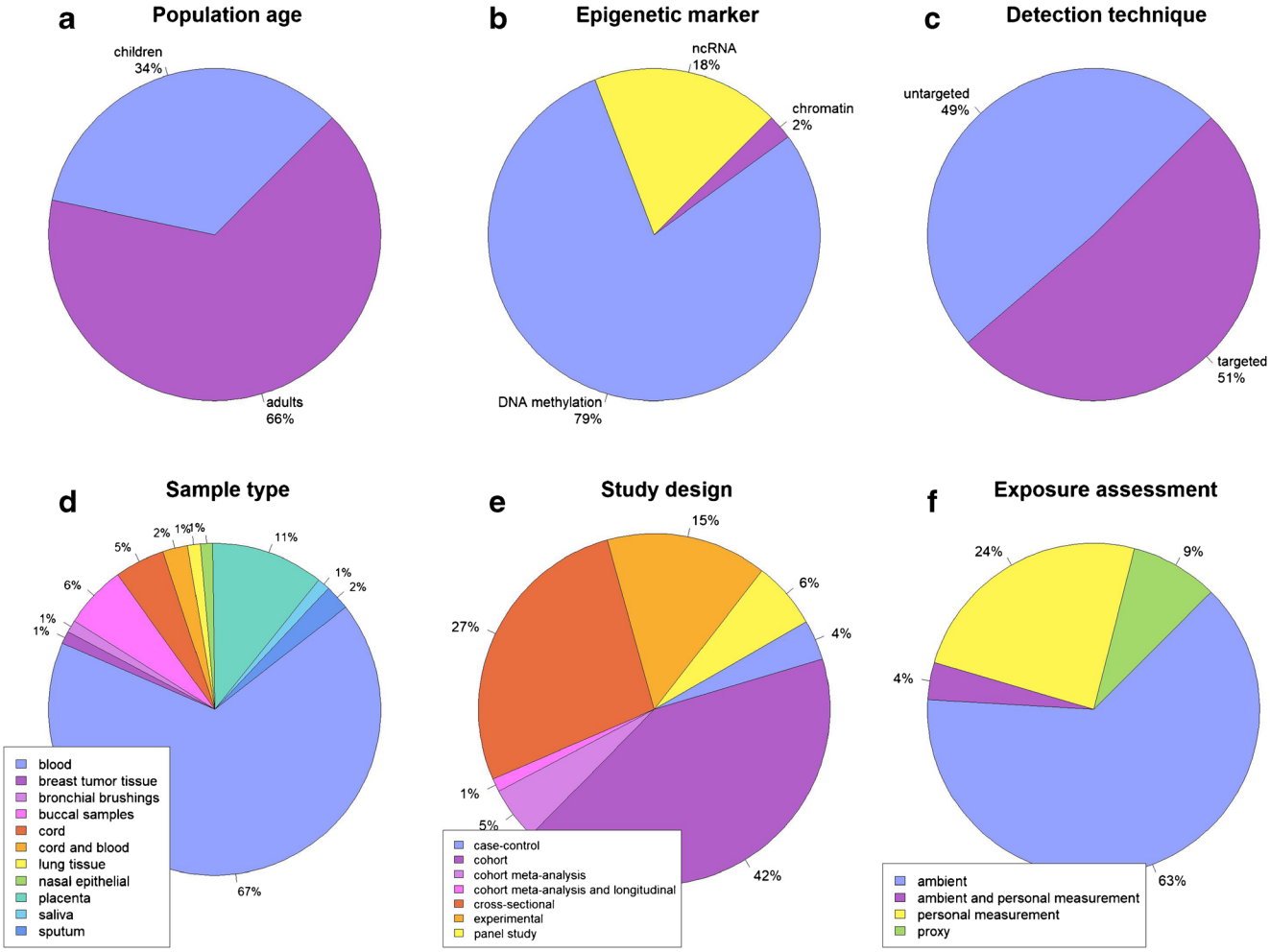


**Fig. 2** Pie charts describe characteristics of the studies included in the review: population age (**a**), epigenetic marker (**b**), detection technique (**c**), sample type used (**d**), study design (**e**) and exposure assessment method (**f**). ncRNA: noncoding RNA

measurement error than smoking, and levels of exposure to air pollution are generally much lower than those related to direct and active inhalation of tobacco smoke. In particular, this work shows that most of the studies on air pollution are performed in countries with relatively low ambient levels of particulate air pollution.

Consistent evidence exists, however, in children and adults for a negative association between overall PM levels and global or L1 methylation, with less consistent findings regarding the methylation of *Alu* repetitive sequences. Methylation of repetitive elements represents almost 50% of global genomic methylation and has, therefore, been often used as proxy of global methylation [108]. However, the discrepancy identified in variations of L1 and *Alu* methylation in relation to air pollution is more in support to the emerging idea that each of these repetitive elements represents a distinct measure of dispersed methylation [109]. Global hypomethylation leads to genomic instability, which is a hallmark of several diseases, including cancer [110].

Effects sizes of air pollution exposure on DNA methylation are generally between 2 and 10%. The respective associations are often highly significant in terms of nominal $p$ values. Since false-positive or false-negative findings in individual studies may arise by chance or bias, many EWASs include a validation strategy, e.g., discovery-replication (albeit not always significant) or a meta-analytical approach in which several studies have been analyzed by consortia (Tables 1 and 2). A consortium approach is powerful but is dependent on the research question and on the availability and compatibility of exposures and outcomes. Generated results are more stable despite population heterogeneity, and consortium analyses typically require a standardized protocol. In the largest birth cohort epigenetics consortium ($n > 29000$), the Pregnancy and Childhood Epigenetics cohort (PACE) [111], a validation at later time points in life is also included. The EXPOsOMICS consortium, albeit smaller than PACE, focuses on air and water pollution measures and includes several cohorts in different life stages, land-use regression modeling (mostly based on the ESCAPE protocols), and personal exposure measurements [112]. Also, other groups fruitfully joined forces, such as the Normative Aging Study and KORA [53]. Although large consortia exist on exposure

and epigenetics, the nested cohorts with available air pollution data remain limited, with the maximum sample size attained so far being 2956 (no. 6, Table 2). There are still opportunities to combine the present studies and build a consortium providing even larger statistical power and including different geographical locations with high- and low-exposure levels.

Another means to increase statistical power in air pollution-methylation associations is by reducing the dimension of the methylome into clusters of genomically proximal CpG sites exhibiting correlated methylation levels and termed as differentially methylated regions (DMRs). Only a few studies implemented DMRs in their studies. One example is a study on short-term and long-term exposures to high levels of CO, $NO_2$, and $PM_{2.5}$ that showed alterations in DMRs of *FOXP3* and to a lower extent *IL-10* [36]. Another study identified using a panel design a larger number of DMRs in 24 h personal monitoring relative to ambient exposure levels of each of $PM_{2.5}$, soot and ultrafine particles [49••].

## Noncoding RNA (miRNA and lncRNA)

The miRNAs that were most frequently identified via agnostic studies were *hsa-miR-30* and *hsa-miR-223* (in 4 studies), while *hsa-miR-21*, *hsa-miR-146a*, and *hsa-miR-222* were the miRNAs most frequently identified via targeted studies (≥ 5 studies). Prominent associated pathways with these miRNAs are cancer [113, 114], hematopoiesis [115], heart disease [116, 117], inflammation, and immune system [118]. The first published study on miRNAs and air pollution was a crossover study on diesel exhaust exposure in 2013 and reported that *miR-21* was significantly downregulated after exposure [94]. Hou and colleagues identified this miRNA in a nontargeted study in truck drivers, and four targeted studies (Table 1) also reported a significant association with particulate matter exposure [89]. *miR-21* is frequently upregulated in tumors and plays a role in the development of heart diseases [119].

Only one study investigated lncRNAs and air pollution [100]. LncRNAs have emerged from obscurity to being recognized as a mechanism of genetic regulation. In cancer biology, the recent application of next-generation sequencing revealed a growing number of lncRNAs whose expression is associated with different cancer types. In addition, the field is moving from annotation of lncRNAs in various tumors toward understanding their importance in key cancer signaling networks [120, 121]. So far, no study has included epitranscriptomics, that is, all functionally relevant changes to the transcriptome that do not involve a change in the ribonucleotide sequence [122].

## Chromatin Regulation

Two studies focused on chromatin remodeling by means of histone modifications. Specific modifications on histone proteins have been linked to chemical exposures such as nickel or arsenic [123] as well as oxidative stress [124] and inflammation [125]. The study of Zheng and colleagues identified in office workers that H3K9ac and H3K36me3 were associated with black carbon exposure [102]. These findings and their role in the pathway from exposure to disease should be further assessed in future studies.

## Multiomics Integration

The identification of DNA methylation, ncRNA, and histone modification signals provides a better understanding of the health risks associated with air pollution such as cardiovascular disease and cancer. More (prospective) studies should, however, be performed to assess their causal role in air pollution-associated outcomes.

Different epigenetic layers can largely interact, and epigenetics may also exhibit reciprocal relations with other biological networks. However, only a few studies included more than one molecular layer of epigenetics or other omics, for example combining DNA methylation with gene expression [49••, 54], with inflammatory protein levels [7, 49••], or with genetic variation [27]. The contribution of different molecular levels to the effects of air pollution warrants further investigation.

## Mitochondrial Epigenetics

Research on mitochondrial epigenetics especially DNA methylation is not as common nor as well understood as nuclear methylation. So far, two studies focused on mitochondrial DNA (mtDNA) methylation: (i) levels in the blood of boiler makers were negatively associated with $PM_{2.5}$ exposure and modified the adverse relationships between $PM_{2.5}$ exposure and heart rate variability outcomes [126]; and (ii) in a mother-newborn cohort, placental mtDNA methylation substantially mediated the association between $PM_{2.5}$ exposure during gestation and mtDNA content in placental tissue, which could reflect signs of mitophagy and mitochondrial senescence [31]. Both studies proved that the mitochondrial methylome is subject to environmental influences and provide important indications to unravel the role of mtDNA methylation.

## Individual Air Pollution Species

Currently, there is a paucity of knowledge about the contribution of individual air particulates. So far, evidence suggests different relative toxicities of PM species: one methylome-wide analysis performed on different sorts of $PM_{2.5}$ in the Normative Aging Study identified a handful of CpG sites associated with Fe, Ni, and V [51], and a targeted study on repair genes and components of $PM_{10}$ in a school showed significant CpG sites associated with acenaphthene, indeno[1,2,3-*cd*]pyrene, and pyrene [37]. Various

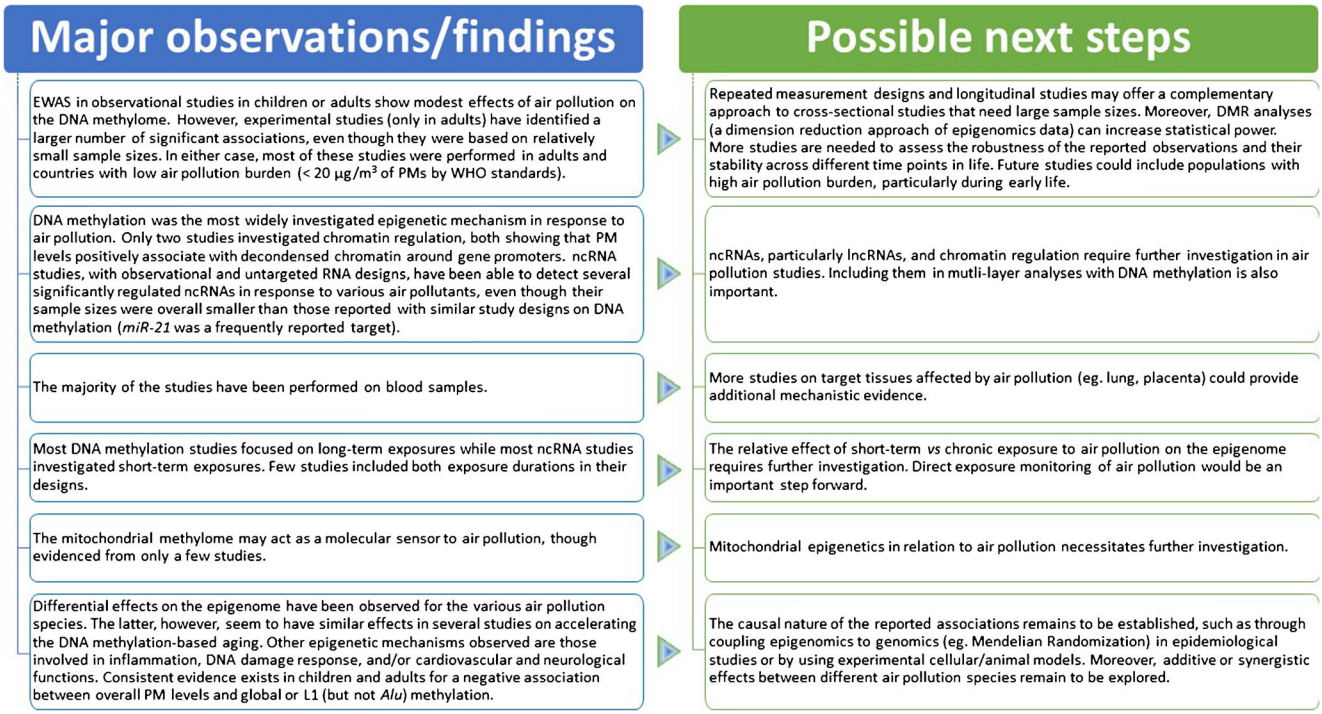


**Fig. 3** Summary of the major observations and findings derived from the review of the literature on the impact of air pollution on epigenetic mechanisms. For every finding, the corresponding possible next step is suggested. DMR: differentially methylated region; EWAS: epigenome-wide association study; ncRNA: noncoding RNA; lncRNA: long noncoding RNA; L1: LINE-1; PM: particulate matter; WHO: World Health Organization

studies observed distinct epigenetic alterations in response to different components of air pollution, including particle size and gases. An EWAS study of the European Prospective Investigation into Cancer and Nutrition (EPIC) on exposure to different particle sizes ($PM_{10}$; coarse PM, subset of $PM_{10}$ that is larger than 2.5 μm; and $PM_{2.5}$), soot (absorbance of the $PM_{2.5}$ filter), $NO_x$, and $NO_2$ described only limited similarity among the CpG sites that are differentially methylated in response to each exposure [54]. The Oxford street study, with an experimental crossover design, revealed different sets of miRNAs associated with each of $NO_2$, ultrafine particles, $PM_{2.5}$, $PM_{10}$, and black carbon [86••]. A targeted study using pyrosequencing of DNA damage and *P53* genes in the ENVIRONAGE cohort included residential $PM_{2.5}$, black carbon, and $NO_2$ exposures and also showed different epigenetic changes in response to these exposures [35].

## Study Design

Most studies had an observational nature, having mainly cohort (42%) or cross-sectional designs (27%), while 12 studies incorporated an intervention. The Oxford street study previously demonstrated the adverse effect of traffic-related exposure to air pollution [127] but recently identified circulating miRNAs involved in the molecular processes of exposure-related diseases [86••]. An exciting result concerning preventative measures was also demonstrated via a human intervention trial demonstrating that acute ambient $PM_{2.5}$-induced DNA methylation changes can be reversed by B-vitamin supplementation [80] as methyl donors. These intervention studies analyzed small sample sizes and low level of exposures and identified heterogeneous epigenetic signals in response to air pollution, thus, limiting the confidence that may be placed on their findings. In this regard, panel studies that allow investigation of repeated measures from the same individuals may be more advantageous to study the biological effect of air pollution, especially for short-term effects. Of note, among the five studies using a panel design, one performed a methylome-wide analysis and found an association between 24-h personal exposure to air pollution and DNA methylation both at single sites and regional clusters [49••].

## Sample Type

The majority of studies have relied on blood or cord blood samples (74%) as surrogate tissues, while some included invasive tissues such as breast or the target tissue being lung. Tissue-/cell-type-specific profiles are particularly important in epigenetic studies because of the driver role epigenetics plays in tissue differentiation and lineage specializing. Caution is recommended in the interpretation of findings as associations of epigenetic profiles with air pollution might be explained by variations in the distribution of different cell types within the analyzed tissue. In this regard, recent advances in

bioinformatics have helped correct for possible changes in cell subpopulations using DNA methylome-based deconvolution algorithms that rely on reference tissues (mostly peripheral, the Houseman algorithm [128]; and cord blood, the Bakulski algorithm [129]) and reference-free methods (a recent but rapidly developing field [130]). However, only half of the studies we reviewed in adults, and even less in children, adjusted findings for cell-type composition or proportion of leucocytes or neutrophils. In addition, changes in minor immune cell subtypes not covered by deconvolution algorithms may also bias DNA methylation analyses' results [131]. Biological matrices that are noninvasive might serve as an excellent source for human samples but are currently underexplored, e.g., exposure-related epigenetic alterations have been recently found in placenta tissues [40, 132], saliva [98, 133], and blood spots [41].

## Conclusion

Over the last decade, considerable progress has been made in environmental epigenetics including the introduction of agnostic studies with accompanying technological advancements. Evidence that exposure to air pollution is linked to epigenetic changes has been provided by several studies; however, most of these biomarkers have not yet been validated and their role in the causal paradigm is not yet clear. An additional challenge would be the integration of multiple epigenetic layers in this rapidly evolving field, which we are just beginning to understand. The major findings and related future directions described above are summarized in Fig. 3. This work provides a timely guide into the existing evidence, the missing gaps, and possible next steps forward.

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### Compliance with Ethical Standards

**Conflict of Interest** Rossella Alfano has a PhD fellowship from Bijzonder Onderzoeksfonds (BOF) Hasselt University.

**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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