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

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**Abstract**

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

**Recent Findings:** 82 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 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), 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 blood stream [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 way 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 [16-18].

Epigenetics is the science of non-genetic 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, non-coding RNA's and chromatin remodelling. These communicating mechanisms ensure the somatically heritable states of gene expression [21]. Epigenetic states display plasticity and are subjected to intrinsic (eg. age, sex, genetic polymorphisms) and extrinsic (eg. environmental exposures and dietary habits) influences [22].

Few reviews (all non-systematic) 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 analysing the existing evidence on the associations between air pollution and the major levels of human epigenetic control: DNA methylation, histone modifications, micro RNA (miRNA) and long non-coding 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 epigenetics changes was made up of four stages, following the PRIMA Statement guidelines [24], as depicted in Fig. 1.

**Fig. 1 here**

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 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, (6) the paper studies ambient air pollution as main exposure or the effects of air pollution trough an experimental design.

In the fourth stage, selected studies were grouped according to epigenetics mechanisms under study investigation and study population; the following information were extracted: authors, country of study, study design, study period, study population (size, age and cohort from which data were derived if available), air pollution exposure under study, epigenetic markers under study along with 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 analysed existing evidence associating air pollution to the major levels of human epigenetic control (Fig.1): DNA methylation (Tables 1-3), non-coding RNAs (Table 4) and chromatin regulation (Table 4). Characteristics of the studies included in the review are summarized in Fig. 2.

**Fig. 2 here**

*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 2-3). All studies among children were observational (cohort, cross-sectional, case-control and longitudinal; Table 1) while adult studies were either observational (cohort, cross-sectional, case-control, longitudinal and panel; Table 2) or experimental (cross-over; 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 (#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 *versus* low polluted areas (#5-6). The first (#5 in South Africa) showed no significant results but was based on a relatively small sample size (n = 21 newborns), while the second (#6 in Czech Republic) reported 9,916 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 $\mu g/m^3$ 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 $\mu g/m^3$ of $PM_{10}$ and are considered relatively lowly polluted, with the exception of one study in Mexico (20 $\mu g/m^3$ of $PM_{10}$; #14), two in China (54 $\mu g/m^3$ of $PM_{10}$; #10 and 15) and one in Iran (60 $\mu g/m^3$ of $PM_{10}$, #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 (#14). All other studies (including the Chinese) investigating the relationship between PM and L1 methylation reported a negative association (#15 and 18), while ozone showed the opposite trend (#18). No association was found for *Alu* methylation with different air pollutants from a study in 11-years-old children (#18), while a positive association was observed with $PM_{10}$ in placenta

(#1). In contrast, global methylation was found always negatively associated with PAH, pyrene (#7) and PM (#6) levels, except from the Iranian study that found a positive association with PM (#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 (#1,14, 16, 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 (#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 (#1-11). Again, all these studies were conducted in relatively low polluted countries (exhibiting < 20 $\mu g/m^3$ of PM based on the WHO Global Urban Ambient Air Pollution 2016 Database). China was the only country identified in the literature (#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 ageing and conducted in two different continents (represented by Germany and USA); this finding is that various air pollution components contribute to accelerated aging (#5, 9 and 10) calculated from DNA methylation levels according Horvath and Hannum methods (using respectively 353 and 71 chronological age-correlated CpGs) [25, 26].

Similarly to studies among children, in adults global methylation estimated from EWAS approaches (#12, 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 (#32). Negative associations with global or L1 methylation were reported for $NO_2$ (#7 and 15), black carbon (#22 and 32) and $SO_4$ (#22) (Table 2). DNA methylation changes have been described for exposure durations to air pollutants ranging from only a few hours (#24) to several years. Longer-term exposures yielded more significant associations (#15). Two studies also investigated global hydroxymethylation levels in relation to PM but reported conflicting findings (#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). Similarly to observations in the cohort studies, one experimental study found a negative association between PM and L1 methylation levels (#6), though another did not find any significant effect (#5). Exposure to fine concentrated ambient particles lowered *Alu* methylation but didn't seem to have an effect on L1 (#7). Though the experimental studies conducted so far using an EWAS approach (#1-4) are based on few subjects ($n \leq 36$), they have identified several CpGs that are significantly altered by $PM_{2.5}$ exposure (#1 and 2) and a large number of CpGs that are differentially methylated in response to diesel exhaust (500 and 2,827 CpGs in #3 and 4, respectively).

*Non-coding RNA (miRNA and lncRNA)*

Only 15 studies investigated the effect of air pollution on non-coding RNAs (ncRNAs) (Table 4), representing only 19% of the literature mined in this work (Fig. 2B). All these studies analysed miRNAs except one that focused on lncRNAs (#15). Five studies had an experimental design (crossover; #1,6 and 8-10), while the remaining ten were observational studies (cohort and cross-sectional). Most of the studies investigated effects of short-term exposure to air pollutants, while fewer focused on chronic exposures including the *in utero* time window (#7,11, and 15). All the studies examined adults, apart from two whose participants were newborns (#11) or school children (#13). Non-coding 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 (RNAseq 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 22-1,630 samples; median = 90; Table 3) relative to those reported with similar study designs on DNA methylation (ranging between 141-2,956 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 (#17) and environmental PM (#16 and 17) exposures on histone modifications in adult blood. One study characterized genome-wide profiles of histone H3 lysine 27 acetylation using chromatin immunoprecipitation sequencing but based on a small sample size (n = 4) while the other study measured candidate global histone H3 modifications (H3K9ac, H3K9me3, H3K27me3 and H3K36me3) *via* ELISA. Both studies showed that PM levels positively associate with a decondensed (i.e. active) chromatin structure around gene promoters, evidence by increased H3K27ac (#16) or decreased H3K27me3 (#17) levels in those regions. Moreover, the $PM_{2.5}$-associated differential H3K27ac markers were enriched in genes involved in immune cell activation (#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 analysed 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 detectible later in life [27]. 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 [28]. One-third of the studies identified in this work were performed in children or in newborns (Fig. 2), 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 1-4), 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 proved to be powerful in identifying and replicating extensive exposure-associated epigenetic alterations, such as maternal tobacco smoke [29], 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 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 [30]. 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 distinct measures of dispersed methylation [31]. Global hypomethylation leads to genomic instability, which is a hallmark of several diseases, including cancer [32].

Effects size of DNA methylation exposure studies are generally between 2-10%. 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 analysed by consortia (Tables 1 & 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 consortium (n >29,000) of the pregnancy and childhood epigenetics cohort (PACE) [33], 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 modelling (mostly based on the ESCAPE protocols) and personal exposure measurements [34]. Also, other groups fruitfully joined forces, such as the Normative Aging Study and KORA [35]. 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 2,956 (#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 *IL10* [36].

*Non-coding 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 [39, 40], hematopoiesis [41], heart disease [42, 43], inflammation and the immune system [44]. 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 [45]. Hou and colleagues identified this miRNA in a non-targeted study in truck drivers, and 4 targeted studies (Table 1) also reported a significant association with particulate matter exposure [46]. *miR-21* is frequently upregulated in tumors and plays a role in the development of heart disease [47].

Only one study investigated long ncRNAs and air pollution [48]. 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 towards understanding their importance in key cancer signaling networks [49, 50]. So far, no study has included epitranscriptomics [51].

*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 [52] as well as oxidative stress [53] and inflammation [54]. The study of Zheng and colleagues identified in office workers that H3K9ac and H3K36me3 were associated with black carbon exposure [55]. These findings and their role in the pathway from exposure to disease should be further assessed in future studies.

*Multi-omics integration*

The identification of DNA methylation, non-coding RNA 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 [56, 57], with inflammatory protein levels [7, 57] or with genetic variation [58]. From these works, it is clear that 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 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 [37], 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 [38]. Both studies proved that the mitochondrial

methylome is subject to environmental influences, and the studies provide important indications to unravel the role of mtDNA methylation.

*Individual air pollution species*

Currently, there is a paucity of knowledge of the individual contribution from different 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 [59], and a targeted study on repair genes and components of $PM_{10}$ in a school study showed significant CpG sites for acenaphthene, indeno[1,2,3-cd]pyrene, and pyrene [60]. Various studies observed distinct epigenetic alterations in response to different components of air pollution, including particles 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 in the results of the different components and mainly observed associations for different CpG sites per exposure [56]. The Oxford street study, an experimental cross-over study, revealed various different miRNAs associated with $NO_2$, ultrafine particles, $PM_{2.5}$, $PM_{10}$ and black carbon [61]. 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 [62].

*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 adverse effect of traffic related exposure to air pollution [63], but recently identified circulating miRNA, involved in the molecular processes of exposure-related diseases [61]. 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 [64] as methyl donors. These intervention studies analysed small sample sizes, 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 5 studies using a panel design, one performed a methylome-wide analysis and found an association between 24-hour personal exposure to air pollution and DNA-methylation both at single sites and regional clusters [57].

*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 analysed 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 [65] and cord blood, the Bakulski algorithm [66]) and reference-free methods (a recent but rapidly developing field [67]). 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 [68]. Biological matrices that are non-invasive might serve as excellent source for human samples but are currently underexplored, e.g. exposure related epigenetic alterations have been recently found in placenta tissues [69, 70], saliva [71, 72], and blood spots [73].

**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.

**Fig. 3 here**

## Compliance with Ethical Standards

### Conflict of Interest

The authors declare that they have no conflict of interest.

### Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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**Figure legends**

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

**Fig. 2.** Pie charts describe characteristics of the studies included in the review: population age (Fig. 2A), epigenetic marker (Fig. 2B), detection technique (Fig. 2C), sample type used (Fig. 2D), study design (Fig. 2E) and exposure assessment method (Fig. 2F).

ncRNA: non-coding RNA.

**Fig. 3. S**ummary of the major observations and findings derived from the review of the litterature 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: non-coding RNA; lncRNA: long non-coding RNA; L1: LINE-1; PM: particulate matter; WHO: World Health Organization.