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Review article

Exposure to ambient air pollution in the first 1000 days of life and alterations in the DNA methylome and telomere length in children: A systematic review

Elena Isaevska [a,*], Chiara Moccia [a], Federica Asta [b], Fabio Cibella [c], Luigi Gagliardi [d], Luca Ronfani [e], Franca Rusconi [f], Maria Antonietta Stazi [g], Lorenzo Richiardi [a]

[a] Department of Medical Sciences, University of Turin, CPO Piemonte, Turin, Italy
[b] Department of Epidemiology, Lazio Regional Health Service, ASL Roma 1, Rome, Italy
[c] Institute for Biomedical Research and Innovation (IRIB), National Research Council of Italy (CNR), Palermo, Italy
[d] Division of Neonatology and Pediatrics, Ospedale Versilia, Viareggio, AUSL Toscana Nord Ovest, Pisa, Italy
[e] Clinical Epidemiology and Public Health Research Unit, Institute for Maternal and Child Health - IRCCS "Burlo Garofolo", Trieste, Italy
[f] Unit of Epidemiology, Meyer Children's University Hospital, Florence, Italy
[g] Center "Behavioral Sciences and Mental Health", Istituto Superiore di Sanità, Rome, Italy

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ABSTRACT

Background: Exposure to air pollution during the first 1000 days of life (from conception to the 2nd year of life) might be of particular relevance for long-term child health. Changes in molecular markers such as DNA methylation and telomere length could underlie the association between air pollution exposure and pollution-related diseases as well as serve as biomarkers for past exposure. The objective of this systematic review was to assess the association between air pollution exposure during pregnancy and the first two years of life and changes in DNA methylation or telomere length in children.

Methods: PubMed was searched in October 2020 by using terms relative to ambient air pollution exposure, DNA methylation, telomere length and the population of interest: mother/child dyads and children. Screening and selection of the articles was completed independently by two reviewers. Thirty-two articles matched our criteria. The majority of the articles focused on gestational air pollution exposure and measured DNA methylation/telomere length in newborn cord blood or placental tissue, to study global, candidate-gene or epigenome-wide methylation patterns and/or telomere length. The number of studies in children was limited.

Results: Ambient air pollution exposure during pregnancy was associated with global loss of methylation in newborn cord blood and placenta, indicating the beginning of the pregnancy as a potential period of susceptibility. Candidate gene and epigenome-wide association studies provided evidence that gestational exposure to air pollutants can lead to locus-specific changes in methylation, in newborn cord blood and placenta, particularly in genes involved in cellular responses to oxidative stress, mitochondrial function, inflammation, growth and early life development. Telomere length shortening in newborns and children was seen in relation to gestational pollutant exposure.

Conclusions: Ambient air pollution during pregnancy is associated with changes in both global and locus-specific DNA methylation and with telomere length shortening. Future studies need to test the robustness of the association across different populations, to explore potential windows of vulnerability and assess the role of the

Abbreviations: CCCEH, Columbia Center for Child's Environmental Health; CHS, Children's Health Study; DOHaD, Developmental Origins of Health and Disease; DMR, Differentially Methylated Region; EDEN, Etude de cohorte généraliste menée en France sur les Déterminants pré et post natals précoces du développement psychomoteur et de la santé de l'Enfant; ENVIRONAGE, ENVIRonmental influence ON early AGEing; EWAS, Epigenome-Wide Association Study; Illumina450K, Infinium HumanMethylation450 BeadChip; LINE1, Long Interspersed Nuclear Element 1; MESH, MEdical Subject Headings; PAH, Polycystic Aromatic Hydrocarbons; PM, Particulate Matter (PM_{10} and $PM_{2.5}$ represent mass concentration of particulate matter with aerodynamic diameter less than 10 μm and 2.5 μm, respectively); qPCR, Quantitative Polymerase Chain Reaction; RE, Repetitive Elements.

* Corresponding author.
E-mail addresses: elena.isaevska@unito.it (E. Isaevska), chiara.moccia@unito.it (C. Moccia), f.asta@deplazio.it (F. Asta), fabio.cibella@ibim.cnr.it (F. Cibella), luigi.gagliardi@uslnordovest.toscana.it (L. Gagliardi), luca.ronfani@burlo.trieste.it (L. Ronfani), franca.rusconi@meyer.it (F. Rusconi), antonia.stazi@iss.it (M.A. Stazi), lorenzo.richiardi@unito.it (L. Richiardi).

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methylation and telomere length as mediators in the association between early exposure to ambient air pollutants and specific childhood health outcomes.

1. Introduction

Air pollution is one of the today's main environmental and public health challenges in both high, and low income countries, with well documented health effects even at low exposure levels (WHO Global Update, 2005). The period during pregnancy and the first years of life is an important window of susceptibility characterized by accelerated growth and developmental plasticity. Epidemiological evidence on the effects of exposures during early life lead to the formation Developmental Origins of Health and Disease (DOHaD) hypothesis, according to which the adaptive responses of the fetus/child to adverse early-life exposures could permanently shape the molecular programming and contribute to later disease predisposition (Gluckman et al., 2008).

Growing evidence links exposure to air pollutants during early life to adverse pregnancy outcomes, including preterm birth (Klepac et al., 2018), reduced lung function, impaired neurodevelopment and susceptibility to later metabolic diseases (Capello and Gaddi, 2018). The biological mechanisms that underlie these associations are still not well understood, although studies suggest that one mechanism may include changes in somatic cell DNA that (1) are influenced by the environment (2) can survive cell replications and (3) have the potential to influence biological processes. The most commonly studied biological markers that satisfy these criteria are DNA methylation and telomere length.

DNA methylation is the most well-known epigenetic mechanism that involves adding a methyl group to the cytosine (C) base of the DNA when next to the guanine (G) base, forming a so called CpG site. The methylation pattern represents a layer of molecular information atop of the DNA sequence that has an important role in wide array of functions, especially in the early embryonic and fetal development, including cell differentiation, regulation of gene expression, imprinting, X-chromosome inactivation and maintenance of genome stability (Dor and Cedar, 2018). The majority of the DNA methylation patterns are established around the period of implantation, making the early gestational period a possible window of susceptibility (Cedar and Bergman, 2012; Slieker et al., 2015). Telomeres, on the other hand, are nucleoprotein complexes located at the end of each chromosome to ensure complete chromosomal replication and prevent genomic instability (Blackburn et al., 2015). Telomere length normally decreases with each cellular replication and variations in telomere length among adults seem to be largely attributed to genetic and environmental determinants that start their effect *in utero* (Entringer et al., 2018a; Okuda et al., 2002; Benetos et al., 2014). Their vulnerability to reactive oxygen species makes them a plausible biomarker, not just for age and cellular replicability, but to for overall exposure to oxidative stress and inflammation. Additionally, they may play an important role mediating the chronic health effects of early-life air pollution exposure (Martens and Nawrot, 2016, 2018; Saenen et al., 2019; Miri et al., 2019).

Several systematic reviews (Desai et al., 2017; Rider and Carlsten, 2019; Luyten et al., 2018; Ferrari et al., 2019) were published on air pollution exposure during the course of life and molecular markers, including early life exposure, but they did not include the majority of the studies on this topic that have been published only recently.

Therefore, our aim was to evaluate the association between exposure to air pollutants during the 1000 days of life, from conception to 2 years, and changes in the DNA methylation patterns and telomere length in children.

2. Methods

Our search strategy included Medical Subject Headings (MESH) terms and keywords based on our population, exposure and outcome of interest (Table 1 and Supplementary Table S1). The exposures of interest were the most commonly measured atmospheric air pollutants: $PM_{2.5}$, PM_{10}, polycyclic aromatic hydrocarbons (PAH), CO, SO_2, NO, NO_2, O_3, volatile organic compounds, black carbon, elemental or organic carbon. The population of interest was restricted to mother-child dyads during the gestational period and children. The outcomes were DNA methylation and telomere length. We limited our search to articles written in English without limitations on the publication date. The search was not restricted to specific exposure assessment methods, tissue sample, and laboratory methods used to measure the outcomes, in order to assess the methodological variability between the selected studies and identify potential gaps in literature.

The literature search in the electronic database PubMed was conducted on October 2020 (Supplementary Table S1) Manual search of the references of the articles selected for full reading and systematic reviews previously published on the topic was also performed to identify additional articles that could match our selection criteria and one was found. Two investigators (EI and CM) conducted the literature search, read all papers and extracted relevant information independently. The discrepancies were resolved by consensus.

From each study that met the eligibility criteria we extracted the following information: study design, country of origin and population size, studied pollutants, method for exposure assessment, concentration levels of the pollutants, studied molecular marker, laboratory technique used to assess the marker, effect estimates for the major findings, covariates considered in the analyses, and relevant results from any additional analyses.

3. Results

3.1. Study characteristics

Our search identified 556 articles; 495 were excluded on the basis of the title or the abstract, and the remaining 61 articles were selected for full reading, Fig. 1. Thirty-two studies met our selection criteria (Lee et al., 2017, 2018, 2020; Feng et al., 2020; He et al., 2018; Abraham et al., 2018; Maghbooli et al., 2018; Nawrot et al., 2018; Plusquin et al., 2018; Perera et al., 2009, 2018; Neven et al., 2018; Yang et al., 2018; Cai et al., 2017; Liu et al., 2019; Martens et al., 2017; Saenen et al., 2017; Gruzieva et al., 2017, 2019; Breton et al., 2016a; Goodrich et al., 2016; Janssen et al., 2013, 2015; Tang et al., 2012; Herbstman et al., 2012; Clemente et al., 2019; Zhou et al., 2019; Song et al., 2019; Nie et al., 2019; Ladd-Acosta et al., 2019; Rosa et al., 2019). All of them were ordered according to publication date (ranging from 2009 to 2020) and summarized in details in Supplementary Table S2.

Thirty articles measured gestational exposures to air pollutants and DNA methylation/telomere length in cord blood/newborn blood or

Table 1
Criteria used to assess the eligibility of the articles.

Study exposure	Particulate Matter ($PM_{2.5}$, PM_{10}), Nitrogen oxides (NO_2, NO), Ozone (O_3), Carbon Monoxide (CO), Sulfur Dioxide (SO_2), Volatile Organic Compounds (VOC), Black Carbon, Elemental carbon, Organic Carbon, Polycyclic Aromatic Hydrocarbons (PAH)
Time of exposure	Pregnancy, the first 2 years of life
Outcome(s)	DNA Methylation, telomere length
Population	Mother/child dyads, children
Study design	Observational studies on singletons.
Time frame	No time frame
Other criteria	Articles in English. No geographical restrictions.

placenta. There is a limited number of studies (n = 5) that investigated the relationship between exposure to air pollution in the first 1000 days of life and DNA methylation/telomere length in childhood (Plusquin et al., 2018; Lee et al., 2018; Gruzieva et al., 2017, 2019; Clemente et al., 2019).

The most commonly studied pollutants were particulate matter (PM) ($PM_{2.5}$ or PM_{10}, 21 studies), NO_2 (10 studies) and PAH (7 studies). Some studies performed trimester specific analyses, other analyzed smaller predefined gestational windows or used distributed-lag model to study weekly exposures during pregnancy. Most of the studies used indirect methods for exposure assessment based on the residential address. Fewer studies measured the exposure by using personal air monitors (Tang et al., 2012; Herbstman et al., 2012; Perera et al., 2009) or by measuring PAH-DNA adducts (Perera et al., 2018; Lee et al., 2017) (cord blood) or PAH metabolites (maternal urine). (Supplementary Table S2).

The articles were based on mother-child dyads from different continents, mostly Europe, North America and Asia. A number of articles included data from the same birth cohort including: eight studies from the ENVIRonmental influence ON early AGEing (ENVIRONAGE) (Nawrot et al., 2018; Plusquin et al., 2018; Neven et al., 2018; Martens et al., 2017; Saenen et al., 2017; Janssen et al., 2013, 2015; Gruzieva et al., 2019), four from the Children's Health Study (CHS) (Gruzieva et al., 2017, 2019; Breton et al., 2016a, 2016b), four from the Etude de cohorte généraliste, menée en France sur les Déterminants pré et post natals précoces du développement psychomoteur et de la santé de l'ENfant (EDEN) cohort (Abraham et al., 2018; Gruzieva et al., 2017, 2019; Clemente et al., 2019), three from Columbia Center for Child's Environmental Health (CCCEH) study (Tang et al., 2012; Herbstman et al., 2012; Perera et al., 2009), three from Chinese cohort from Zhengzhou (Feng et al., 2020; He et al., 2018; Zhou et al., 2019) and two articles from birth cohorts enrolled before and after closing of a coal plant in China (Perera et al., 2018; Lee et al., 2017). Four studies (Plusquin et al., 2018; Gruzieva et al., 2017, 2019; Clemente et al., 2019) meta-analyzed data from multiple European and American birth cohorts.

We classified each of the thirty-two studies into at least one of the following categories: (1) studies of global methylation patterns, (2) studies on candidate-gene methylation that focus on targeted genes of interest, usually with a hypothesized role in the association between ambient air pollution exposure and human diseases (3) epigenome-wide association studies (EWAS) that used untargeted methylation analysis of thousands of CpGs across the genome to discover unknown associations between air pollutants and CpG methylation and (4) telomere length studies.

Twenty-five studies focused on DNA methylation, Tables 2-3. Of them, ten measured global DNA methylation (Table 2) using different methods, including quantifying total genomic methylation and measuring the methylation in repetitive elements (RE), such as LINE1 and/or Alu. In this group we additionally included two studies did not measure global methylation based on traditional methods, but summarized methylation data across all loci targeted on the Infinium HumanMethylation450 BeadChip (Illumina450K platform). This platform is mainly used in EWAS studies (Table 4) where it provides a cost-efficient measurement of DNA methylation of more than 450 thousand CpGs across the entire genome. The CpGs included in the platform account for 2% of the total genomic CpG content, but are enriched with potentially relevant CpGs clustered near transcription start sites (called CpG islands) and in the body of the majority of human genes. EWAS studies used CpG-based, region-based approach (differentially methylated region, DMR analysis) and/or enriched pathway analysis to discover associations between air pollutants and untargeted CpGs or regions across the genome. Candidate gene methylation (n = 12 studies) was estimated either by pyrosequencing or by using CpG data from EWAS studies, Table 3. Seven studies analyzed telomere length (Table 5) by using the quantitative polymerase chain reaction (qPCR) protocol developed by Cawthon and expressed the telomere length as relative T/S ratio (Cawthon, 2002).

3.1.1. *Findings in newborn blood, cord blood and placenta*

3.1.1.1. Global DNA methylation studies. Table 2 summarizes the main findings of the studies that assessed the link between air pollution during pregnancy and global methylation patterns. Most studies reported global loss of methylation following increased gestational exposure to $PM_{2.5}$ and PM_{10}, Liu et al., 2019; Cai et al., 2017; Breton et al., 2016a; Janssen et al., 2013 mostly due to exposures in the first trimester.

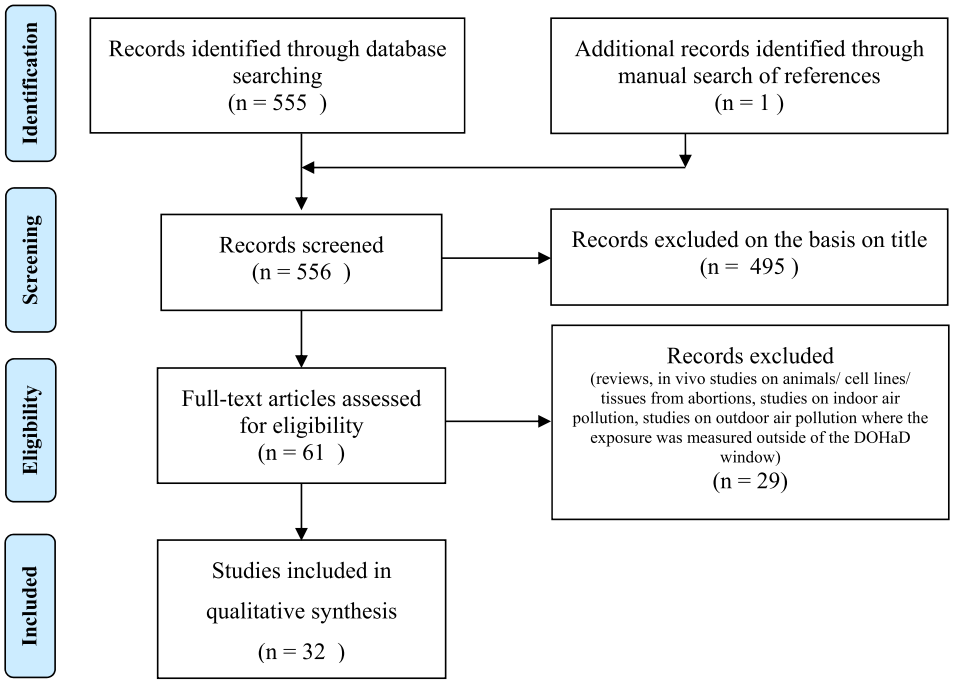

Fig. 1. Flow chart describing the selection of the articles.

Table 2
Studies on air pollution exposure during pregnancy and global DNA methylation.

Author	Method	Sample	Pollutant	Main findings
Liu et al., 2019	LINE1	Cord blood, n = 258	PM_{10}, $PM_{2.5}$ and PM_1 (ambient air)	PM_{10}, $PM_{2.5}$ and PM_1 exposure was associated with ↓LINE1-DNAm. The analyses were conducted only for the period between 12 and 20 gestational week, previously identified as window of exposure associated with preterm birth.
Ladd-Acosta et al., 2019	Illumina 450K	Placenta, n = 124; cord blood n = 163	NO_2, O_3 (ambient air)	O_3 associated with ↓DNAm at open sea regions (in cord blood) and shelf regions (in both cord blood and placenta). O_3 associated with ↑DNAm at CpG islands (in placenta) and shore regions (both cord blood and placenta). NO_2 associated with ↓DNAm in placenta, mostly at CpG islands and shore regions.
Abraham et al., 2018	LINE1, Alu, Illumina 450K	Placenta, n = 668	PM_{10}, NO_2 (ambient air)	No association, except PM_{10} exposure the day before birth and ↑Alu-DNAm.
Maghbooli et al., 2018	HPLC	Placenta, n = 92	$PM_{2.5}$, PM_{10} (ambient air)	Exposure to $PM_{2.5}$ and PM_{10} in T1 positively correlated with global DNAm
Yang et al., 2018	LINE1, Alu	Cord blood, n = 106	PAH (maternal urine)	PAH (measured only in T3) associated with ↓Alu and ↓LINE1-DNAm
Cai et al., 2017	LINE1	Placenta, n = 181	PM_{10} (ambient air)	PM_{10} exposure in T1 associated with ↓LINE1-DNAm, mostly in newborns with FGR
Lee et al., 2017	LINE1	Cord blood, n = 217	PAH (cord blood DNA adducts)	PAH-DNA adducts associated with ↓LINE1-DNAm
Breton et al., 2016a	LINE1, AluYb8	Newborn blood; n = 392 in LINE1, n = 181 in AluYb8 analyses	$PM_{2.5}$, O_3, PM_{10}, NO_2 (ambient air)	PM_{10} and O_3 exposure in T1 associated with ↓LINE1-DNAm. O_3 exposure in T3 associated with ↑LINE1-DNAm
Janssen et al., 2013	LC/MS-MS	Placenta, N = 240	$PM_{2.5}$ (ambient air)	$PM_{2.5}$ associated with ↓global DNAm, mostly driven by exposures in T1 (during implantation)
Herbstman et al., 2012	ELISA-based	Cord blood, N = 168	PAH (ambient air, maternal urine)	Ambient PAH (measured only in T3) associated with ↓global DNAm.

Abbreviations: DNAm: DNA methylation; T1, T2 and T3: first, second and third trimester, respectively; Illumina450K: Illumina's Infinium HumanMethylation450K BeadChip; HPLC: High Performance Liquid Chromatography; LC/MS-MS: Liquid chromatography coupled with tandem mass spectrometry. ELISA: enzyme-linked immunosorbent assay; LINE1: Long Interspersed Nuclear Element 1; PM: Particulate Matter; PAH: Polycystic Aromatic Hydrocarbons.

Exposure to PAH was also associated with decreased global loss of methylation in cord blood. The exposure to PAH was measured only at one time point (mainly during late pregnancy), and therefore data on other potential important windows of exposure are lacking (Yang et al., 2018; Lee et al., 2017; Herbstman et al., 2012). The findings regarding O_3 and NO_2 were less conclusive (Breton et al., 2016a; Ladd-Acosta et al., 2019).

3.1.1.2. Candidate genes studies. Exposure to air pollution was associated with altered methylation (mostly, but not exclusively, with gene-promoter hypermethylation, Supplementary Table S3) of a number of targeted genes with names reported in Table 3. No gene was analyzed in more than one study. Briefly, exposure to air pollutants (the studied pollutant and analyzed tissue are shown in brackets) was associated with altered methylation of several genes involved in pre-eclampsia (NO_2; placenta) (Abraham et al., 2018), circadian rhythm regulation ($PM_{2.5}$; placenta) (Nawrot et al., 2018), growth (PM_{10}, NO_2, SO_2; cord blood) (He et al., 2018), obesity (PM_{10}, NO_2, SO_2; cord blood) (Feng et al., 2020), DNA repair and tumor suppression ($PM_{2.5}$, BC; placenta) (Neven et al., 2018), glucocorticoid metabolism (PM_{10}; placenta) (Cai et al., 2017), energy regulation ($PM_{2.5}$; placenta) (Saenen et al., 2017), pro-allergic immune responses (PAH; cord blood) (Tang et al., 2012), synthesis of antioxidant enzymes ($PM_{2.5}$, NO_2; cord blood) (Gruzieva et al., 2017), and mitochondrial functions ($PM_{2.5}$, NO2, cord blood, placenta). (Janssen et al., 2015; Zhou et al., 2019).

3.1.1.3. Epigenome-wide association studies. Table 4 describes the main findings of the studies that conducted an epigenome wide analysis (Abraham et al., 2018; Plusquin et al., 2018; Gruzieva et al., 2017, 2019; Goodrich et al., 2016; Ladd-Acosta et al., 2019; Perera et al., 2009; Breton et al., 2016b). The largest study on NO_2 exposure meta-analyzed data from 1508 mother-child dyads from nine separate cohorts from Europe and United States by measuring epigenome-wide methylation in cord blood (Gruzieva et al., 2017). The top three CpGs associated with gestational NO_2 exposure were mapped to genes important for mitochondrial functions. One of the CpGs (cg08973675, in the SLC25A28 gene) showed similar direction of association in an another cohort of newborns (Ladd-Acosta et al., 2019) (Supplementary Table S3). Gestational NO_2 exposure was also associated with placental methylation, in CpGs and DMRs mainly mapped to genes linked to preeclampsia (Abraham et al., 2018) and inflammatory processes. (Ladd-Acosta et al., 2019) (Supplementary Table S3).

Particulate matter exposure in pregnancy was assessed in four epigenome-wide studies (Abraham et al., 2018; Plusquin et al., 2018; Gruzieva et al., 2019; Breton et al., 2016b). The largest and most recent study conducted by Gruzieva and colleagues (Gruzieva et al., 2019) studied cord blood methylation and included information on 1949 and 1551 mother-child dyads in the corresponding PM_{10} and $PM_{2.5}$ analyses (Gruzieva et al., 2019). The study reported associations between gestational PM_{10} or $PM_{2.5}$ exposures and the methylation of 20 CpGs in cord blood. The robustness of the associations of the 6 PM_{10}-related CpGs was tested in an independent cohort of newborns and only the PM_{10}-related CpG, cg18640183 in the P4HA2 gene showed consistent direction of association. (Supplementary Table S3). The region-based analysis identified large number of DMRs related to the studied air pollutants. Two PM_{10}-related DMRs in the genes H19 and MARCH11 replicated in the another cohort of newborns. Other two studies on particulate matter and DNA methylation in newborns (Plusquin et al., 2018; Breton et al., 2016b) had smaller sample size and/or were based on cohorts already included in the meta-analysis by Gruzieva and colleagues.

Only one epigenome-wide study estimated PAH exposure (Perera et al., 2009). The study was published in 2009 and used a slightly older method to perform unbiased methylation profiling. The top finding was the change in methylation of the ACSL3 gene in relation to PAH exposure.

3.1.1.4. Telomere length studies. The association between air pollution exposure in pregnancy and telomere length at birth was assessed in seven studies (Perera et al., 2018; Martens et al., 2017; Clemente et al., 2019; Song et al., 2019; Nie et al., 2019; Rosa et al., 2019; Lee et al., 2020). The main findings are presented in Table 5. The studies were

Table 3
Studies on air pollution exposure during pregnancy and candidate gene DNA methylation.

Author	Candidate gene	Method	Sample	Pollutant	Main findings
Feng et al., 2020	GPR61 gene	QMS-PCR	Cord blood, n = 568	PM_{10}, NO_2, SO_2 (ambient air)	PM_{10} and SO_2 exposure in pregnancy was associated with ↓GPR61-DNAm, while NO_2 exposure with ↑GPR61-DNAm.
Zhou et al., 2019	SOD2 gene	QMS-PCR	Cord blood, n = 568	PM_{10}, NO_2, SO_2 (ambient air)	PM_{10} in T2 associated with ↑DNAm, NO_2 in T3 with ↓DNAm.
He et al., 2018	H19 gene	QMS-PCR	Cord blood, n = 527	PM_{10}, NO_2, SO_2 (ambient air)	SO_2 exposure in pregnancy was associated with ↓DNAm in H19 promoter, while PM_{10} and $NO_2$with ↑DNAm.
Abraham et al., 2018	Genes with specific expression patterns in the placenta (18972 CpGs in total)	Illumina 450K	Placenta, n = 668	PM_{10}, NO_2 (ambient air)	NO_2 associated with ↓DNAm in ADORA2B, CAPN10 and with ↑DNAm in PXT1/KCTD20. PM_{10} associated with ↑DNAm of SLC44A5, ADCK5 and TMG6 genes and ↓DNAm in KYNU and TUBGCP2.
Nawrot et al., 2018	Circadian pathway genes: CLOCK, NPAS2, BMAL1, CRY1, CRY2, PER1, PER2, PER3	pyro	Placenta, n = 407	$PM_{2.5}$ (ambient air)	$PM_{2.5}$ associated with ↑BMAL1-DNAm. T1 exposure with ↓CLOCK-DNAm. T3 and LM exposure with ↑NPAS2 and CRY1-DNAm and ↓PER2 and PER3-DNAm.
Lee et al., 2018	GSTP1 gene	pyro	Nasal epi-thelia at 7 years, n = 131	$PM_{2.5}$ (ambient air)	$PM_{2.5}$ exposure >37 gestational weeks associated with ↑DNAm in GSTP1.
Neven et al., 2018	DNA repair and tumor suppressor genes:APEX1, OGG1, PARP1, ERCC1, ERCC4, p53, DAPK1	pyro	Placenta, n = 463	$PM_{2.5}$, BC, NO_2 (ambient air)	$PM_{2.5}$ associated with ↑DNAm in APEX1, OGG1, ERCC4 and p53 gene and with ↓DNAm of DAPK1 gene. BC was associated with ↑DNAm in APEX1, PARP1 and ERCC4 gene. NO_2: no associations.
Gruzieva et al., 2017	Antioxidant and inflammatory genes (38 genes in total, 739 CpGs)	Illumina 450K	Cord blood, n = 1508	NO_2 (ambient air)	NO_2 exposure in pregnancy was associated with ↑DNAm in CAT gene and ↓DNAm in TPO gene
Cai et al., 2017	Fetal growth related genes: HSD11B2 and NR3C1	pyro	Placenta, n = 181	PM_{10} (ambient air)	Exposure in T1 and T2 associated with ↑DNAm in HSD11B2 gene
Saenen et al., 2017	LEP gene	pyro	Placenta, n = 361	$PM_{2.5}$ (ambient air)	$PM_{2.5}$ exposure in T2 associated with ↓LEP-DNAm
Janssen et al., 2015	Mitochondrial DNA regions: D-loop and MT-RNR1 region	pyro	Placenta, n = 381	$PM_{2.5}$ (ambient air)	$PM_{2.5}$ exposure, mostly in T1, was associated with ↑mtDNAm in both D-loop and MT-RNR1 region
Tang et al., 2012	Asthma-related genes: IFNγ and IL4	BGS	Cord blood, n = 53	PAH (ambient air)	PAH measured in T3 associated with ↑ IFNγ-DNAm

Abbreviation: DNAm: DNA methylation; T1, T2, T3 and LM: first, second, third trimester and last month of pregnancy, respectively; pyro: pyrosequencing; BGS: Bisulfite Genomic Sequencing; Illumina450K: Illumina's Infinium HumanMethylation450K BeadChip; QMS-PCR: Quantitative Methylation-Specific-Polymerase Chain Reaction; DNAm: DNA methylation; PM: Particulate Matter; PAH: Polycystic Aromatic Hydrocarbons.

generally consistent in reporting an inverse association between gestational exposure to air pollutants (mainly PM or PAH) and telomere length in newborn blood and placenta (Perera et al., 2018; Martens et al., 2017; Song et al., 2019; Nie et al., 2019; Rosa et al., 2019), although there were studies that also reporter longer telomeres in later pregnancy (Martens et al., 2017; Rosa et al., 2019).

3.1.2. *Findings in children*

The number of studies that conducted analysis in children is limited. Briefly, an association was found between late gestation $PM_{2.5}$ exposure and nasal epithelia methylation of a candidate gene (GSTP1 gene, involved in xenobiotic metabolism) at age 7, with strongest effects seen in boys (Lee et al., 2018). A large meta-analysis on early life air pollution and telomeres included more than 1300 8-year old children from six European birth cohort studies reported that prenatal exposure and exposure during the first year of life to $PM_{2.5}$ and NO_2 was associated with shorted telomeres at age 8 (Clemente et al., 2019). There were no studies in children exploring the association between early-life exposure to air pollution and global methylation.

The two large meta-analyses on $PM^{48}_{2.5/10}$ and NO^{35}_2 exposure, described previously, tried to replicate the association seen in newborns in several cohorts of older children, in order to see whether they are stable throughout childhood. The results were inconclusive. Only one NO_2-related CpG cg08973675 in the SLC25A28 gene showed robust association in two cohorts of older children aged 4 and 8 years. Out of total 20 CpGs associated with PM_{10} or $PM_{2.5}$, none showed clear association across different cohorts of older children (6 PM_{10} related CpGs were tested in three cohorts of children aged 7–9 years and two cohorts of children aged 15–16 years, while the 14 $PM_{2.5}$-related CpGs were tested in two cohorts of children aged 7–9 years and in one cohort of teenagers aged 15–16 years). It should be noted that 3 PM_{10} associated CpGs (cg00905156, cg06849931 and cg06849931 mapped to three genes important for respiratory health: FAM13A, NOTCH4 and P4HA2 gene, respectively) showed consistent direction in at least one of the three independent cohorts 7-9 year-olds.

4. Discussion

The studies included in this review provided evidence that prenatal exposure to air pollutants is linked with global and locus-specific alterations in DNA methylation as well as telomere length shortening in newborn cord blood and placenta. Further studies are needed to elucidate whether these changes can influence childhood outcomes years after the exposure. The number of studies that studied air pollution exposure during the first 1000 days of life by measuring DNA methylation or telomere length in older children was limited.

Global loss of methylation is linked with genomic instability and can predispose to the development of human diseases (Pogribny and Beland, 2009). Gestational exposure to air pollutants ($PM_{2.5/10}$ and PAH) was generally associated with global loss of methylation in different cohorts and different tissues (placenta and cord/newborn blood), independently of the exposure assessment method and the laboratory method used to measure the global methylation patterns. Some studies on PM exposure that conducted trimester-specific analyses, identified the beginning of the pregnancy as a potential period of susceptibility (Cai et al., 2017; Breton et al., 2016a; Janssen et al., 2013). It is plausible that exposures in early pregnancy might be strongly associated with global loss of methylation since the period around the implantation is the period when the epigenetic reprogramming occurs *de novo* methylation takes place (Cedar and Bergman, 2012). Exposure to air pollutants in such vulnerable period might interfere with the DNA methylation machinery and lead to generalized loss of methylation (Teneng et al., 2011). Weather these changes persist into childhood is unknown.

Air pollution is believed to influence human health through the

Table 4
Studies on air pollution exposure during pregnancy and epigenome-wide methylation patterns.

Author	Sample	Pollutant	Main findings
Ladd-Acosta et al., 2019	Cord blood, n = 163; placenta, n = 124	NO_2, O_3 (ambient air)	Several DMRs associated with NO_3 and O_3, some of which were sex specific. The DMRs in the placenta seemed to be tissue-specific, while those reported in cord blood, showed similar direction in the placenta.
Gruzieva et al., 2019, [a]	Discovery analyses in cord/newborn blood: n = 1949 (PM_{10}) and n = 1551 ($PM_{2.5}$). Replication analyses in cord blood (n = 688), peripheral blood of 7–9yrs (n1 = 692, n2 = 525 n3 = 901) and 15–16yrs (n1 = 198, n2 = 903)	$PM_{2.5}$, PM_{10} (ambient air)	Gestational exposure to ether $PM_{2.4}$ or PM_{10} was associated with 20 CpGs at birth and hundreds of DMRs. Enriched pathways: NOTCH signaling pathway, Rho GTPase cycle, neurotransmitter release cycle, GABA synthesis, release, reuptake and degradation. Two DMRs (H19 and MARCH1) showed consistent direction in an independent cohort of newborns. Three CpGs cg00905156 (FAM13A), cg06849931 (NOTCH4) and cg18640183 (P4HA2) showed consistent association in at least one of the independent cohorts of older children aged 7–9.
Abraham et al., 2018	Placenta, n = 668	PM_{10}, NO_2 (ambient air)	Out of the 4 identified PM_{10} or NO_2-related CpGs, 2 were in the ADORA2B gene linked with hypoxia and pre-eclampsia. Strongest association was seen after exposures in second trimester. More than 20 DMRs were also identified.
Gruzieva et al., 2017, [a]	Discovery analyses in cord/newborn blood, n = 1508. Replication analyses in peripheral blood of 4 yrs (n = 733) and 8 yrs (n = 786)	NO_2 (ambient air)	Gestational NO_2 exposure was associated with 3 CpG sites in mitochondria-related genes: cg12283362 (LONP1), cg24172570 (HIBADH), and cg08973675 (SLC25A28). Enriched pathways: negative regulation of cellular process, negative regulation of biological process and integrin-linked kinase signaling pathway. The cg08973675 replicated in an independent sample of older children.
Goodrich et al., 2016	Cord blood, n = 22	NOx (ambient air)	No CpG passed the FDR threshold. Enriched pathways were found related to xenobiotic metabolism, oxygen and gas transport, and sensory perception of chemical stimuli
Perera et al., 2009	Cord blood, n = 22	PAH (ambient air)	Top finding was the ACSL3 gene whose association with PAH was further confirmed in a slightly larger sample (N = 53)

Two studies by Plusquin et al., 2018 and Breton et al., 2016b were excluded from the main summary of the findings since they included cohorts included in a meta-analysis by Gruzieva et al., 2019.
All studies, except for Perera et al., 2008 (that used Methylation Sensitive Restriction Fingerprinting), used Illumina's Infinium HumanMethylation450K BeadChip to assess epigenome wide methylation patterns. Abbreviations: DMRs: Differentially Methylated Regions; FDR: False Discovery Rate; PM: Particulate Matter; PAH: Polycystic Aromatic Hydrocarbons.
[a] The study included children in their replication analysis.

Table 5
Studies on exposure to air pollution during pregnancy and/or 1st year of life and telomere length.

Author	Sample	Pollutant	Conclusion
Lee et al., 2020	Cord blood, n = 152	$PM_{2.5}$ (ambient air)	Exposures during pregnancy was associated with ↓TL, mostly due to exposures in mid-gestation between 12 and 20 gestational week.
Clemente et al., 2019,a	Peripheral blood, 8 yrs; n = 1396	NO_2, $PM_{2.5}$ (ambient air)	Gestational NO_2 exposure was associated with shorter TL across all trimesters. 1 year-childhood exposure to NO_2 and $PM_{2.5}$ was associated with shorter TL.
Song et al., 2019	Cord blood, n = 743	$PM_{2.5}$, PM_{10}, SO_2, CO, NO (ambient air)	Exposures to $PM_{2.5}$, PM_{10}, CO, and SO_2 during third trimester were related to shorter TL. Associations were stronger in males.
Nie et al., 2019	Cord blood, n = 247	PAH (maternal urine)	Association with shorter TL.
Rosa et al., 2019	Cord blood, n = 423	$PM_{2.5}$ (ambient air)	Exposure during gestational weeks 4–9 associated with shorter TL. Exposure during weeks 14–19 and 34–36 associated with longer TL. Associations were stronger in girls.
Perera et al., 2018	Cord blood, n = 225	PAH (ambient air)	Association with shorter TL.
Martens et al., 2017	Cord blood, n = 698; placenta, n = 660	$PM_{2.5}$ (ambient air)	Exposure during mid-gestation (weeks 12–25 for cord blood and weeks 15–27 for placenta) associated with shorter TL. Exposure in late pregnancy (weeks 32–34) associated with longer telomeres in cord blood. No effect modification by sex.

All studies used the same method for estimating telomere length (quantitative polymerase chain reaction). Abbreviations: TL: telomere lenght, PM: Particulate Matter; PAH: Polycystic Aromatic Hydrocarbons.
[a] The study was the only one conducted in children. It was also the only study where exposure after pregnancy was assessed, in particular, during the first year of life.

generation of reactive oxygen species, as increased oxidative stress is known to trigger number of redox-sensitive cellular signaling pathways (Kelly, 2003). Although, the heterogeneity of the published candidate

gene studies (all studies analyzed different sets of genes with different sets of pollutants in different tissues) did not provide enough evidence to draw strong conclusions regarding a specific gene, the overall findings suggest that gestational exposure to pollutants can lead to methylation changes in cord blood and placenta, in genes involved in key cellular responses to oxidative stress (Neven et al., 2018; Gruzieva et al., 2017; Janssen et al., 2015; Zhou et al., 2019), and genes with known role in growth, early life development and pregnancy disorders (He et al., 2018; Abraham et al., 2018; Cai et al., 2017; Saenen et al., 2017).

Epigenome-wide association studies independently tested the association between gestational air pollution exposure and more than 400 000 CpGs throughout the genome. This agnostic approach allows to identify novel genomic regions associated with the exposure. The strongest and most robust associations were seen for CpGs or DMRs mapped to genes with roles in mitochondrial (Gruzieva et al., 2017; Ladd-Acosta et al., 2019), respiratory functions (Gruzieva et al., 2019) and fetal growth (Gruzieva et al., 2019). The rest of the CpGs were mapped to genes with known roles in auto-immunity (Ladd-Acosta et al., 2019), inflammation (Ladd-Acosta et al., 2019), inter/intracellular signaling (Gruzieva et al., 2019; Ladd-Acosta et al., 2019), cell cycle regulation (Gruzieva et al., 2019; Ladd-Acosta et al., 2019), embryonal development and adverse pregnancy outcomes (Abraham et al., 2018; Gruzieva et al., 2019; Breton et al., 2016b). Gestational exposure to PM_{10} and altered methylation in the NOTCH signaling pathway with an important role in embryonal development, while (Gruzieva et al., 2019) gestational NO_2 exposure was associated with altered methylation in pathways that downregulate cellular functions and are involved in cell migration, proliferation and survival (Gruzieva et al., 2017).

These findings compliment those from global methylation and candidate gene studies, and provide further evidence that gestational air pollution exposure can have an impact on early-life global and locus-specific methylation patterns. The gestational period, especially early pregnancy, is the period when the DNA methylation pattern undergo most dramatic changes: active and passive de-methylation of nearly all maternal and paternal patterns, process of epigenome-wide re-methylation and, finally, gene-specific changes that initiate embryonal cell differentiation (Cedar and Bergman, 2012). Since the majority of these patterns are believed to be largely maintained in the next cell replications it is possible that air pollution exposure during this dynamic period can leave epigenetic fingerprints that might influence later health and disease outcomes.

It should be noted however, that the identified CpGs/DMRs/enriched pathways were quite heterogeneous between studies and it seems difficult to replicate the EWAS findings across different populations. This could be partially explained by pollutant-specific effects that trigger different biological cascades, as suggested by the lack of overlap between the top NO_2-related (Gruzieva et al., 2017) and PM_{10}-related CpGs (Gruzieva et al., 2019) and the different enriched pathways found in the NO_2 and PM_{10} analyses. Particulate matter-specific effects might be even more difficult to replicate due to the possible differences in the source and chemical composition of the particulate matter particles in different populations, although this probably is not the major cause. In the context of air pollution, environmental mixtures and different confounding pattern across study populations may be contributing factors to baseline differences in laboratory conditions, unmeasured batch effects and different pre-processing pipelines (Breton et al., 2017; Pekkanen and Pearce, 2001). For example, different studies use different methods of estimating pollutant concentration and misclassification of exposure is possible when studying exposure based on residential address. Two PM_{10}-related DMRs (Gruzieva et al., 2019) (including the imprinted growth-related gene H19, that showed associations with prenatal PM_{10} exposure in a previous candidate gene study (He et al., 2018)) showed promising results by replication in an independent cohort of newborns. This could mean that future studies should consider expanding the search from single CpG level to genomic regions that contain multiple CpGs, or even to epi-signatures based on methylation levels of hundreds of CpGs spread across the genome to find patterns predictive of the exposure. However, due to the relatively small effect sizes and the variable chemical composition of PM, advanced statistical methods would be needed to appropriately model the exposure (or the concurrent exposure to multiple pollutants that would better reflect real-life exposure), as well as large sample size, in order to detect robust associations on population level that could accurately predict early-life exposure to pollution, as was previously done with prenatal exposure to smoke (Richmond et al., 2018). Future studies should also assess whether the changes seen at birth are stable throughout childhood. Moreover, it is known that methylation patterns are tissue-specific. For example, cord blood and placenta are expected to have different methylation patterns due to their different biological function and cell composition. According to one study (Ladd-Acosta et al., 2019), DMRs identified in cord blood showed consistent direction of effect in the placental tissue, while the DMRs identified in placenta seem tissue-specific. Further studies are needed to confirm these findings.

Telomere length at birth is a reflection of the complex interplay between genetics, number of cell divisions (dependent of both somatic growth and gestational age), exposure to oxidative stress and the counter-regulatory effect of the telomerase (Entringer et al., 2018b). Findings from studies included in this review indicate that prenatal exposure to air pollution can lead to telomere attrition, as seen at birth and in childhood. It is known that the variability in telomere length in adults most likely originates *in utero* and that short telomeres in adults are associated with higher risk for chronic-non communicable diseases. Therefore, the possible effect of prenatal exposure to air pollution on early telomere maintenance system might not be negligible when talking about the lifetime risk of chronic non-communicable diseases (Entringer et al., 2018b). Results regarding possible windows of exposure during pregnancy and the effect modification by sex are unclear. The authors of two studies that reported longer telomeres in late gestation hypothesized that prolonged exposure to air pollution might increase activity of the telomerase (Martens et al., 2017; Rosa et al., 2019).

It is known that DNA methylation and the telomere maintenance system are interrelated on cellular lever (Saenen et al., 2019; Martens and Nawrot, 2016). This is especially true during the early gestational period. Short telomeres in embryonic cells might led to downregulation of the *de novo* DNA methyl transferases, that in turn might induce genomic instability and impair embryonic stem cell differentiation. DNA methylation is can also influence telomere length via the regulation of the telomerase activity (Harrington and Pucci, 2018; Joyce et al., 2018).

Considering the both DNA methylation and the telomere system are key players in many cellular functions, future studies need to assess their potential to leave long-term consequences in the context of the fetal origins of health and disease hypothesis. Unfortunately, only few studies included in this review analyzed data in relation to some specific birth or childhood outcomes. Some of them provided preliminary evidence, that global methylation, methylation at specific CpGs and/or genes and telomere length, might mediate the association between prenatal exposure to air pollution and birth outcomes (Cai et al., 2017; Liu et al., 2019), childhood respiratory outcomes (Lee et al., 2018; Perera et al., 2009; Breton et al., 2016b) and neurodevelopmental scores (Perera et al., 2018; Nie et al., 2019), respectively.

In conclusion, prenatal exposure to air pollution was associated with global loss of methylation, telomere shortening and epigenetic alterations mapped to key genes involved in oxidative stress response, mitochondrial function, inflammation, fetal growth and development. Additional studies are needed to test the robustness of the associations across different populations and explore potential windows of vulnerability during pregnancy and early-life, as well as to confirm the role of DNA methylation and telomere length as mediators in the association between prenatal and early-life exposure to air pollution and later childhood outcomes.

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Authors' contributions

Screened and selected the articles: EI and CM. Designed search strategies: all authors. Extracted the data: EI and CM. Wrote the first draft of the manuscript: EI. Critically reviewed the manuscript for important intellectual content: all authors. Read and approved the final version: all authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2020.110504.

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