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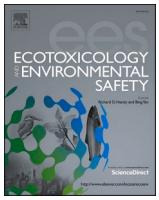

# Prenatal and early-life air pollutant exposure and epigenetic aging acceleration

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ABSTRACT

*Background:* This study investigated the association of prenatal and early childhood exposure to air pollution with epigenetic age acceleration (EAA) at six years of age using the Environment and Development of Children Cohort (EDC Cohort)

*Materials & methods:* Air pollution, including particulate matter [< 2.5 μm ($PM_{2.5}$) and < 10 μm ($PM_{10}$) in an aerodynamic diameter], nitrogen dioxide ($NO_2$), ozone ($O_3$), carbon monoxide (CO), and sulfur dioxide ($SO_2$) were estimated based on the residential address for two periods: 1) during the whole pregnancy, and 2) for one year before the follow-up in children at six years of age. The methylation levels in whole blood at six years of age were measured, and the methylation clocks, including Horvath's clock, Horvath's skin and blood clock, PedBE, and Wu's clock, were estimated. Multivariate linear regression models were constructed to analyze the association between EAA and air pollutants.

*Results:* A total of 76 children in EDC cohort were enrolled in this study. During the whole pregnancy, interquartile range (IQR) increases in exposure to $PM_{2.5}$ (4.56 μg/$m^3$) and CO (0.156 ppm) were associated with 0.406 years and 0.799 years of EAA (Horvath's clock), respectively. An IQR increase in $PM_{2.5}$ (4.76 μg/$m^3$) for one year before the child was six years of age was associated with 0.509 years of EAA (Horvath's clock) and 0.289 years of EAA (Wu's clock). $PM_{10}$ (4.30 μg/$m^3$) and $O_3$ (0.003 ppm) exposure in the period were also associated with EAA in Horvath's clock (0.280 years) and EAA in Horvath's skin and blood clock (0.163 years), respectively.

*Conclusion:* We found that prenatal and childhood exposure to ambient air pollutants is associated with EAA among children. The results suggest that air pollution could induce excess biological aging even in prenatal and early life.

## 1. Introduction

According to the global burden of disease study, air pollution is the first-ranked environmental risk factor attributable to seven million deaths worldwide yearly (World Health Organization, 2016). The detrimental health effects of air pollution have been widely reported, including particulate matter with an aerodynamic diameter < 2.5 μm ($PM_{2.5}$) and < 10 μm ($PM_{10}$), nitrogen dioxide ($NO_2$), ozone ($O_3$), carbon monoxide (CO), and sulfur dioxide ($SO_2$). Especially children and fetuses are particularly vulnerable to air pollution because of their growing status and physiological traits. These air pollutants can affect the structural and functional growth of organ systems from the fetal stage

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through early, middle, and late childhood (Miller and Marty, 2010). Moreover, children are exposed to higher doses than adults owing to their faster respiration rate and a relatively larger volume of air intake per kilogram of body weight (United States Environmental Protection Agency, 2011). The health effects of air pollution on children and fetuses have been widely reported, including low birth weight, preterm birth (Stieb et al., 2012), infections and allergic symptoms (Brauer et al., 2002), respiratory function (Gehring et al., 2013), and brain development and cognitive impairment (Lopuszanska and Samardakiewicz, 2020).

Unlike epidemiologic solid evidence on the association between air pollution exposure in prenatal or childhood, the biological mechanisms are not fully understood. The suggested mechanisms related to the health effects of air pollution include direct oxidative effects of O3 or induction of reactive oxygen species. Systemic inflammation and reactive oxygen species have been proposed. PM2.5 is especially known for its systemic inflammation because it can enter the circulatory system via inhalation. Other pollutants, such as NO, were linked to systemic inflammation among students (Patel et al., 2013). Toxicological research suggests air pollutants could induce the production of reactive oxygen species (De Kok et al., 2006); recent research has indicated that the pathways relating air pollution to health may entail epigenetic modifications (Rider and Carlsten, 2019).

"Epigenetic clocks" are developed based on the hypothesis that the methylation levels of a set of specific CpG sites can predict the chronological age (Horvath and Raj, 2018). This cellular aging manifests in various forms, including alterations in the DNA telomere and repair process, chromosomes, RNA, transcriptome, metabolism, oxidative stress, mitochondrial function, cell senescence, and inflammation (Xia et al., 2017). Biological markers of aging can detect cellular-level changes before the manifestation of evident phenotypic alterations, offering insights into the aging process across a lifespan (Levine et al., 2018). Recently, epigenetic age acceleration (EAA), the discrepancy between the epigenetic methylation age and the chronological age, has been suggested as a biological marker of aging (Van der Laan et al., 2022). The discrepancy between biological and epigenetic ages, particularly when the epigenetic age exceeds the biological age significantly, is recognized as a risk factor for disease development and mortality in middle-aged or older adults (Fransquet et al., 2019). Recently, the epigenetic changes related to air pollution exposure were studied as a marker of aging. However, most studies examined EAA in adulthood, except for one study which reported an association between childhood exposure to indoor air pollution and EAA at the age of seven (de Prado-Bert et al., 2021; White et al., 2019). Nevertheless, there is no evidence of an association between EAA in children, which could be associated with potential disease status in later life via aging in methylation, and prenatal and early-life exposure to air pollutants. From an ethical and preventive standpoint, studying the health effects of particulate matter on children allows for early identification and intervention, which can mitigate long-term health consequences and promote better overall health trajectories. This preventative approach benefits not only the immediate well-being of children but also contributes to the broader public health goal of reducing chronic diseases and improving quality of life across the lifespan. Furthermore, children provide a unique opportunity to study the health impacts of particulate matter due to their relative "clean slate" at birth, free from the environmental and lifestyle confounders that accumulate in adults, such as smoking, occupational hazards, and long-term dietary habits. Despite having no control over air pollution, children are significantly affected by it, highlighting the need for focused research on this demographic. Therefore, this study aimed to investigate the association between exposure to air pollution and EAA in childhood, using the Environment and Development of Children Cohort (EDC cohort).

## 2. Materials and methods

### 2.1. Study participants

EDC cohort is an ongoing prospective birth cohort including 726 mother–child pairs enrolled during pregnancy and followed up every two years (Kim et al., 2018). Briefly, environmental exposures were assessed during pregnancy using biological samples and a questionnaire, and the birth outcomes were assessed using the medical records. The information on the demographic factors, medical history, and environmental factors were surveyed through the follow-up examination. Among these participants, 80 children at age six were analyzed for DNA methylation from the whole blood as a sub-cohort. Four children who were unavailable to assess the air pollutant exposure during pregnancy due to a lack of home address information during pregnancy were excluded. The Institutional Review Board of Seoul National University Hospital approved the study protocol (IRB No. 1201–010–392), and informed consent was obtained from all parents.

### 2.2. Exposure assessment

The average concentration levels of air pollutants, including $PM_{2.5}$, $PM_{10}$, $NO_2$, $O_3$, CO, and $SO_2$, were calculated for two periods: 1) during the whole pregnancy and 2) during one year leading up to the follow-up assessment when the children were six years old. The $PM_{2.5}$, $PM_{10}$, $NO_2$, $O_3$, CO, and $SO_2$ levels were obtained from publicly accessible data provided by AIRKOREA (http://www.airkorea.or.kr/eng), which is an initiative that provides hourly air pollution concentrations measured at 257 monitoring stations nationwide, operated by the Korea Ministry of Environment. As nationwide monitoring of $PM_{2.5}$ levels launched in 2015, the $PM_{2.5}$ levels during the whole pregnancy (2009–2011) were estimated by the chemical transport model, Community Multiscale Air Quality (CMAQ, version 4.7.1), with the metrological model, Weather Research and Forecast (version 3.3.1) and the emission inventories, Sparse Matrix Operator Kernel Emission models (version 3.1) (Skamarock and Klemp, 2008). The methods and validity of $PM_{2.5}$ modelling data are described in the published articles (Han et al., 2020; Kim et al., 2017a, 2017b). For each participant, the hourly air pollutant levels were estimated based on their residential addresses, which were determined using ArcGIS by calculating the Euclidean distance between each address and the nearest monitoring station.

### 2.3. DNA methylation

DNA methylation levels were analyzed in the blood samples collected from the EDC cohort children around six years old (± 1 month). The obtained samples were stored at −70°C, and transferred with the cold chain system to Macrogen (Seoul, Korea), where the DNA methylation data were processed. DNA methylation data was analyzed by using the Illumina Infinium HumanMethylation 450 K BeadChip (Illumina, San Diego, CA, USA). A detailed description of the experimental procedures was published previously (Choi et al., 2023). For quality control, array CpG probes with a detection *P*-value exceeding 0.05 in more than 25 % of the samples were removed. Subsequently, the filtered data were normalized using the Beta Mixture Quantile (BMIQ) method (Teschendorff et al., 2013) and corrected for batch effects using the ComBat package in R. Cell type distribution (the fraction of CD8+T cells, CD4+T cells, natural killer (NK) cells, B cells, monocytes, and neutrophils) was estimated using Minfi R package (Aryee et al., 2014).

### 2.4. Epigenetic age acceleration

This study used Horvath's clock (Horvath, 2013a), Horvath's skin and blood clock (Horvath et al., 2018), PedBE (McEwen et al., 2020), and Wu's clock algorithm (Wu et al., 2019). In the studies on children and childhood exposure, Horvath's clock was reported as the most

widely used epigenetic clock (Musci et al., 2023). The PedBE clock is relatively newer but was developed using data from 0 to 20 years of age. Wu's clock was established to predict DNA methylation levels among children. The epigenetic clocks were selected with reference to the previous study on the association between the early life exposome and EAA in children (de Prado-Bert et al., 2021). All methylation-based age prediction model was calculated using the *methylclock* R package (Pelegí-Sisó et al., 2021). Briefly, these algorithms use information on the methylation status at empirically selected CpG sites related to chronological aging and calculate the estimated DNA methylation age. The Horvath's, Horvath's skin and blood, PedBE, and Wu's clocks use 353 CpGs, 391 CpGs, 94 CpGs, and 111 CpGs, respectively (Supplementary Table S1). EAA, an acceleration (or deviation) between the age predicted from DNA methylation age and the chronological age [(the date of examination - the date of birth) / 365.24], has been proposed as a biomarker for aging and age-related disease (Fransquet et al., 2019). EAA was calculated from the residuals obtained after regressing the chronological age and DNA methylation age adjusted for the cell counts. The cell type distribution was used to construct the regression model to calculate the EAA. They were estimated by using the Housman algorithm from the DNA methylation profiles using R package *meffil* as the fraction of CD8+T cells, CD4+T cells, natural killer (NK) cells, B cells, monocytes, and neutrophils (Aryee et al., 2014; Houseman et al., 2012; Min et al., 2018).

### 2.5. Covariates

The covariates were selected based on a literature review, including sex (girls and boys), multiple births (singleton and multiple birth), season of birth (spring, summer, fall, and winter), maternal age at birth (≤ 29, 30–34, and ≥ 35 years), maternal education (≤ high school, college, and > college), maternal pre-pregnancy body mass index (normal [<23 kg/m$^2$], overweight [23–24.9 kg/m$^2$], and obese [≥25 kg/m$^2$]), and birthweight (<2500, 2500–3499, 3500–3999, and ≥4000 g), and preterm birth (gestational age < 37, and ≥ 37 weeks).

### 2.6. Statistical analysis

The Pearson's correlation between chronological age and epigenetic age were investigated. Multivariate linear regression models were constructed to assess the association between exposure to air pollutants ($PM_{2.5}$, $PM_{10}$, $NO_2$, $O_3$, CO, and $SO_2$ during the whole pregnancy and for one year before the follow-up at six years), and the EAA values were calculated using Horvath's clock, Horvath's skin and blood clock, PedBE, and Wu's clock. The covariates included the child's sex, multiple births, season of birth, maternal age at birth, maternal education level, maternal pre-pregnancy body mass index, birthweight, and preterm birth. Additionally, we performed a sensitivity analysis limited to singleton births. A generalized additive model was constructed to show the nonlinear associations between air pollutants and EAA. If a significant association between air pollutants and EAA in the epigenetic clock was found, the relationships of DNA methylation values were also evaluated at each CpG probe in the epigenetic clock and air pollutants, adjusting the maternal age at birth, maternal education level, birth weight, gestational age, multiple births, and season of birth. A false discovery rate (FDR) correction was performed to account for multiple tests. All analyses were conducted using the *methylclock* package and R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). *P*-values (two-sided) < 0.05 were regarded as statistically significant.

## 3. Results

Among 76 included children, 39 (51.3 %) were female and 37 (48.7 %) were male (Table 1); multiple births accounted for 9.2 %. The season of birth was mostly spring (55.3 %), followed by summer (25.0), winter (17.1), and fall (2.6). The maternal age at birth was mostly 30–34 years (56.6 %), followed by ≤ 29 years (31.6 %) and ≥ 35 years (11.8 %). Most mothers were college graduates (71.1 %) with a normal BMI (55.3 %). Children with low birth weights accounted for 7.9 %, and 11.8 % were preterm births.

**Table 1**
Characteristics of the study participants.

| Variables | n (%) |
|---|---|
| ***Sex*** | |
| Girls | 39 (51.3) |
| Boys | 37 (48.7) |
| ***Multiple birth*** | |
| Singleton | 69 (90.8) |
| Multiple birth | 7 (9.2) |
| ***Season of Birth*** | |
| Spring | 42 (55.3) |
| Summer | 19 (25.0) |
| Fall | 2 (2.6) |
| Winter | 13 (17.1) |
| ***Maternal age at birth (years)*** | |
| ≤29 | 24 (31.6) |
| 30–34 | 43 (56.6) |
| ≥35 | 9 (11.8) |
| ***Maternal education*** | |
| High school or below | 13 (17.1) |
| College | 54 (71.1) |
| Above college | 9 (11.8) |
| ***Maternal pre-pregnancy BMI (kg/m$^2$)*** | |
| Normal (<23) | 42 (55.3) |
| Obese (23–24.9) | 22 (28.9) |
| Obesity (≥25) | 12 (15.8) |
| ***Birthweight (g)*** | |
| <2500 | 6 (7.9) |
| 2500–3499 | 46 (60.5) |
| 3500–3999 | 19 (25.0) |
| ≥4000 | 5 (6.6) |
| ***Gestational age (weeks)*** | |
| < 37 weeks | 9 (11.8) |
| ≥ 37 weeks | 67 (88.2) |

The average concentrations (± SD) of $PM_{2.5}$ (μg/m$^3$), $PM_{10}$ (μg/m$^3$), $NO_2$ (ppm), $O_3$ (ppm), CO (ppm), and $SO_2$ (ppm) during the whole pregnancy were 33.35 (± 3.37), 56.31 (± 10.43), 0.034 (± 0.007), 0.024 (± 0.007), 0.522 (± 0.136), and 0.005 (± 0.002), respectively (Table 2. 3). The mean concentrations of $PM_{2.5}$ (μg/m$^3$), $PM_{10}$ (μg/m$^3$), $NO_2$ (ppm), $O_3$ (ppm), CO (ppm), and $SO_2$ (ppm) exposed to children during one year before follow-up at age six were 25.10 (± 2.67), 46.86 (± 4.39), 0.031 (± 0.004), 0.023 (± 0.002), 0.542 (± 0.063), and 0.005 (± 0.001), respectively.

The mean epigenetic ages (± SD) measured by Horvath's clock, Horvath's skin and blood clock, PedBE clock, and Wu's clock were 5.79 (± 1.21), 4.03 (± 0.58), 5.08 (± 0.30), and 3.58 (± 0.63) years, respectively. Supplementary Figure S1 shows the significant correlation between chronological age and DNA methylation age. The EAA values calculated using Horvath's clock, Horvath's skin and blood clock, PedBE clock, and Wu's clock were 0.00004 (± 1.11), −0.02 (± 0.52), −0.002 (± 0.26), and 0.004 (± 0.59), respectively.

The linear regression results showed significant associations between air pollutants and EAA (Table 4). An IQR increase in $PM_{2.5}$ and $PM_{10}$ during one year before follow-up at six years was associated with 0.509 (95 % CI = 0.043 – 0.975) and 0.280 (95 % CI = 0.029 – 0.530) years increase in EAA in Horvath's clock. An IQR increase in $PM_{2.5}$ at six years was also associated with EAA in Wu's clock of 0.289 (95 % CI = 0.030 – 0.548) years. An IQR increase in the one-year average of CO before the six-year follow-up was associated with 0.337 (95 % CI = 0.029 – 0.646). $PM_{2.5}$ exposure during pregnancy was associated with the EAA in Horvath's clock, as an IQR increase in $PM_{2.5}$ with 0.406 (95 % CI = 0.031 – 0.782) years of EAA. An IQR increase in CO exposure during the whole pregnancy was associated with Horvath's clock and Horvath's skin and blood clock with 0.779 (95 % CI = 0.362 – 1.196) and 0.393 (95 % CI = 0.200 – 0.586) years of EAA. These associations were also observed in

**Table 2**
Distribution of the air pollutant concentrations.

| | Mean | SD | Min | 25th | Median | 75th | Max |
|---|---|---|---|---|---|---|---|
| ***Average during the Whole Pregnancy*** | | | | | | | |
| $PM_{2.5}$ (μg/m$^3$) | 33.35 | 3.37 | 24.94 | 30.87 | 33.3 | 35.43 | 44.27 |
| $PM_{10}$ (μg/m$^3$) | 56.31 | 10.43 | 29.19 | 49.57 | 53.55 | 61.86 | 87.77 |
| $NO_2$ (ppm) | 0.034 | 0.007 | 0.016 | 0.030 | 0.035 | 0.038 | 0.046 |
| $O_3$ (ppm) | 0.024 | 0.007 | 0.011 | 0.018 | 0.023 | 0.028 | 0.038 |
| CO (ppm) | 0.522 | 0.136 | 0.250 | 0.430 | 0.499 | 0.586 | 0.974 |
| $SO_2$ (ppm) | 0.005 | 0.002 | 0.002 | 0.004 | 0.005 | 0.006 | 0.011 |
| ***Average during one year before the follow-up at age six*** | | | | | | | |
| $PM_{2.5}$ (μg/m$^3$) | 25.10 | 2.67 | 21.37 | 22.84 | 23.72 | 27.6 | 30.11 |
| $PM_{10}$ (μg/m$^3$) | 46.86 | 4.39 | 39.92 | 44.1 | 45.81 | 48.4 | 60.93 |
| $NO_2$ (ppm) | 0.031 | 0.004 | 0.017 | 0.029 | 0.032 | 0.034 | 0.037 |
| $O_3$ (ppm) | 0.023 | 0.002 | 0.020 | 0.022 | 0.023 | 0.025 | 0.032 |
| CO (ppm) | 0.542 | 0.063 | 0.403 | 0.500 | 0.541 | 0.577 | 0.721 |
| $SO_2$ (ppm) | 0.005 | 0.001 | 0.004 | 0.005 | 0.005 | 0.006 | 0.009 |

EAA, epigenetic age acceleration; SD, standard deviation.

**Table 3**
Distribution of Epigenetic age and EAA in years.

| | Mean | SD | Min | 25th | Median | 75th | Max |
|---|---|---|---|---|---|---|---|
| ***Horvath's clock*** | | | | | | | |
| Epigenetic age | 5.79 | 1.21 | 3.81 | 4.94 | 5.63 | 6.55 | 10.05 |
| EAA | 0.00004 | 1.11 | −2.12 | −0.73 | −0.15 | 0.49 | 3.46 |
| ***Horvath's skin and blood clock*** | | | | | | | |
| Epigenetic age | 4.03 | 0.58 | 3.00 | 3.57 | 3.99 | 4.29 | 6.41 |
| EAA | −0.02 | 0.52 | −0.84 | −0.36 | −0.04 | 0.29 | 2.04 |
| ***PedBE clock*** | | | | | | | |
| Epigenetic age | 5.08 | 0.30 | 4.41 | 4.87 | 5.05 | 5.24 | 5.86 |
| EAA | −0.002 | 0.26 | −0.46 | −0.18 | −0.02 | 0.16 | 0.77 |
| ***Wu's clock*** | | | | | | | |
| Epigenetic age | 3.58 | 0.63 | 2.37 | 3.15 | 3.50 | 3.96 | 5.71 |
| EAA | 0.004 | 0.59 | −1.30 | −0.41 | −0.14 | 0.47 | 2.07 |

The EAA was calculated after adjusting for cell counts.
EAA, epigenetic age acceleration; SD, standard deviation.

the nonlinear models constructed using GAM (Fig. 1).

Sensitivity analysis results among singleton births conducted (Supplementary Table S1). An IQR increase in $PM_{2.5}$ during pregnancy was associated with 0.564 (95 % CI = 0.218 – 0.910) years increase in EAA in Horvath's clock. $O_3$ exposure during pregnancy showed a significant association with 0.558 (95 % CI = 0.137 – 0.979) years increase in EAA in Horvath's clock. For the period of one year before age six, an IQR increase in $PM_{2.5}$ was associated with an increase of 0.278 (95 % CI = 0.020 – 0.536) years in Wu's clock. $O_3$ exposure during the same period showed significant associations with increases in EAA of 0.192 (95 % CI = 0.040 – 0.345) years in Horvath's skin and blood clock, and 0.220 (95 % CI = 0.039 – 0.401) years in Wu's clock.

The x-axis shows the change of each air pollutant, and the y-axis shows the difference from the mean EAA calculated using each epigenetic clock. The generalized additive model was constructed with the adjustment for the maternal age at birth, maternal education level, birth weight, gestational age, multiple births, and season of birth. EAA, epigenetic age acceleration; IQR, interquartile range

Among the significant associations between air pollutants and EAA in DNAm clocks, this study also examined the relationship between air pollutants and CpG site methylation patterns across CpG sites used to calculate DNAm age (Supplementary Table S2) but found no significant associations after considering multiple hypothesis testing using FDR (Supplementary Table S3).

## 4. Discussion

### 4.1. Main findings

This study examined the association of exposure to air pollution during pregnancy and childhood with EAA at the children's age of six years using seventy-six mother–child dyads in the EDC cohort. During the whole pregnancy, an IQR increase in exposure to $PM_{2.5}$ (4.56 μg/m$^3$) was associated with 0.406 years of EAA in Horvath's clock. An IQR increase in exposure to CO (0.16 ppm) was associated with 0.779 years of EAA in Horvath's clock and 0.393 years of EAA in Horvath's skin and blood clock. During childhood exposure at six years old, an IQR increase in $PM_{2.5}$ (4.76 μg/m$^3$) was associated with EAA in Horvath's clock (0.509 years) and Wu's clock (0.289 years), and $PM_{10}$ was associated with EAA in Horvath's clock (0.280 years). $O_3$ exposure in childhood was also significantly associated with EAA in Horvath's skin and blood clock (+0.163 years by +0.009 ppm). The results of the sensitivity analysis suggest that the observed associations were substantially influenced by the birth conditions (singleton vs. twin). Hence, air pollution exposure in the prenatal and early life periods may lead to excess biological aging. To our knowledge, this is the first report on a significant association between ambient air pollution exposure and EAA in children.

### 4.2. Air pollution and methylation aging: previous research

In our study, EAA in 6 years old was significantly associated with prenatal exposure to $PM_{2.5}$, and postnatal exposure to $PM_{10}$. The association between additional aging in methylation clocks and air pollution was consistently reported in previous research, although the study population was usually older adults. The study using the Cooperation for Health Research in the Region of Augsburg (KORA) cohort data, comprising 1777 participants with a mean age of 61 years, found that a rise of one interquartile range (0.97 μg/m$_3$) in $PM_{2.5}$ was linked with an approximately 0.3-year increase in age acceleration as measured by the

**Table 4**
Association between air pollutants and EAA.

| Exposure | Horvath's clock | Horvath's skin and blood clock | PedBE clock | Wu's clock |
|---|---|---|---|---|
| ***Pregnancy*** | | | | |
| $PM_{2.5}$ (μg/$m^3$) | **0.406 (0.031 – 0.782)*** | 0.109 (−0.071 – 0.290) | −0.035 (−0.132 – 0.062) | −0.051 (−0.267 – 0.165) |
| $PM_{10}$ (μg/$m^3$) | 0.322 (0.002 – 0.642) | 0.04 (−0.115 – 0.195) | 0.030 (−0.053 – 0.113) | 0.035 (−0.149 – 0.219) |
| $NO_2$ (ppm) | 0.154 (−0.186 – 0.494) | 0.11 (−0.049 – 0.268) | −0.021 (−0.107 – 0.065) | 0.109 (−0.080 – 0.297) |
| $O_3$ (ppm) | 0.387 (−0.144 – 0.918) | 0.119 (−0.133 – 0.371) | −0.021 (−0.156 – 0.115) | −0.001 (−0.302 – 0.300) |
| CO (ppm) | **0.779 (0.362 – 1.196)*** | **0.393 (0.200 – 0.586)*** | −0.005 (−0.120 – 0.111) | 0.219 (−0.031 – 0.469) |
| $SO_2$ (ppm) | 0.096 (−0.238 – 0.430) | 0.065 (−0.092 – 0.222) | −0.032 (−0.116 – 0.052) | 0.168 (−0.014 – 0.351) |
| ***One year, before age six*** | | | | |
| $PM_{2.5}$ (μg/$m^3$) | **0.509 (0.043 – 0.975)*** | 0.131 (−0.094 – 0.356) | −0.007 (−0.128 – 0.115) | **0.289 (0.030 – 0.548)*** |
| $PM_{10}$ (μg/$m^3$) | **0.280 (0.029 – 0.530)*** | 0.11 (−0.009 – 0.229) | −0.016 (−0.082 – 0.049) | 0.061 (−0.083 – 0.205) |
| $NO_2$ (ppm) | −0.169 (−0.463 – 0.125) | −0.075 (−0.214 – 0.064) | −0.005 (−0.080 – 0.069) | −0.114 (−0.277 – 0.050) |
| $O_3$ (ppm) | 0.254 (−0.085 – 0.592) | **0.163 (0.006 – 0.320)*** | −0.052 (−0.138 – 0.033) | 0.161 (−0.027 – 0.349) |
| CO (ppm) | 0.337 (0.029 – 0.646)* | 0.131 (−0.017 – 0.278) | 0.004 (−0.077 – 0.084) | 0.100 (−0.077 – 0.277) |
| $SO_2$ (ppm) | 0.874 (−2.103 – 3.852) | −0.154 (−1.559 – 1.251) | −0.039 (−0.789 – 0.712) | 0.164 (−1.499 – 1.827) |

Adjusted for maternal age at birth, maternal education level, birth weight, gestational age, multiple births, and season of birth.
EAA, epigenetic age acceleration; IQR, interquartile range.
* *P*-value < 0.05.

Horvath clock (Ward-Caviness et al., 2016). In the study using the data from 589 older males in a Normative Aging Study, a 1 μg/$m^3$ increase in the one-year average $PM_{2.5}$ was linked to a 0.52-year increase in the EAA of the Horvath clock and suggested that 20 CpG sites contributed to the increase in EAA (Nwanaji-Enwerem et al., 2016). Alexandra et al. evaluated 2747 women with an average age of 57 years for the association between air pollutants and EAA using Hannum's, Horvath's, and Levine's clocks. However, some studies have also reported non-significant associations between air pollution and EAA. Among a cohort of a cohort of 2747 Caucasian adults, the one-year average $PM_{2.5}$, $PM_{10}$, and $NO_2$ concentrations were not significantly associated with EAA, but $PM_{2.5}$ was significantly and positively related with EAA in the subgroups divided by the $PM_{2.5}$ component composition, where $PM_{2.5}$ may have originated from wood smoke and surface soil (White et al., 2019). In our study, prenatal CO exposure and childhood $O_3$ exposure were also significantly associated with EAA. Research on this topic is limited; however, a study suggests that prenatal exposure to O3 during pregnancy may influence methylation of the long interspersed nuclear elements (LINE1) in the blood of newborns, potentially impacting future health outcomes (Breton et al., 2016). However, other air pollutants did not show significant associations with EAA in our study. A study with 1508 participants in four European and American birth cohorts reported different DNA methylation level in mitochondria-related genes by prenatal exposure to $NO_2$ (Gruzieva et al., 2017). These differences could be derived from the fact that air pollutants are highly correlated and have the potential for interaction. Wanying et al. measured personal airborne chemical exposure (including volatile organic compounds and polycyclic aromatic hydrocarbons) for three days a month for five consecutive months. They investigated their association with DNA methylation clocks (including Hannum clock, Horvath clock, DNAm Phenoage, DNAm Grim age, and DNAm estimator of the telomere length) in 73 healthy older adults. They reported that multiple airborne chemical exposure is significantly associated with the DNAm Phenoage (Shi et al., 2022).

The epigenetic changes related to development and aging may simply reflect the dynamic control of DNA methylation associated with maintenance and repair (Bock et al., 2012), which suggests that there are more vulnerable CpG sites for air pollutants than other CpG sites. It is more plausible that several CpG sites among all affected CpG sites are the key linking air pollution and health effects. DNA methylation in the specific CpG sites and air pollution have also been investigated. In 2016, the association between prenatal exposure to traffic-related air pollution, using $NO_2$, and epigenome-wide cord blood methylation was investigated, and three CpG sites related to the mitochondria were significantly associated with exposure to prenatal air pollution (Gruzieva et al., 2017). Similarly, there were significant associations between PM exposure in the prenatal period and methylation in two CpG sites (FAM13A, cg00905156; NOTCH4, cg06849931), which play a significant role in lung function and asthma in newborn samples. Sbihi et al. reported that prenatal exposure to $NO_2$, traffic-related air pollution, was associated with EAA in the gestational period and allergen sensitization at one year old. They concluded that EAA could mediate between prenatal air pollution exposure and atopy (Sbihi et al., 2019).

### 4.3. Meaning of methylation aging in children

EAA has been studied as a predictor of the mortality risk in several studies. Riccardo et al. estimated that a five-year higher EAA is associated with a 21 % higher all-cause mortality risk using four longitudinal cohorts of older people including Lothian Birth Cohorts 1921 and 1936, the Framingham Heart Study, and the Normative Aging Study (Marioni et al., 2015). Recently, a systematic review and meta-analysis of the association between EAA and mortality risk reported the pooled effects across seven studies as five years of EAA associated with an 8–15 % mortality risk (Fransquet et al., 2019). On the other hand, these studies examined older adult populations, so it is difficult to assume that children with EAA are also at a high mortality risk. In childhood, EAA is considered a risk factor for accelerated aging and allergy diseases. Suarez et al. examined the relationship of EAA with weight-for-age and height-for-age among 239 Finnish adolescents aged 11.0–13.2 years, and revealed a significant relationship between EAA and excess growth than what was expected at their age. Cheng et al. reported that EAA is associated with increased total serum IgE levels, atopic sensitization, and current asthma among 408 children with a mean age of 7.8 years. A cohort study with 145 Canadian mother-child dyads reported that EAA in the gestational period was significantly associated with early-life allergen sensitization at one year old, as measured by a skin prick test (Sbihi et al., 2019).

### 4.4. Air pollutants and aging

Age-associated phenotypes, such as hair greying and skin wrinkling could share components that may be driven by a chronological clock via the underpinning epigenome function (Field et al., 2018). Hannum et al. reported that the expression of genes related to chronological age was strongly associated with age-associated methylation markers used in Hannum's clock. (Hannum et al., 2013). If EAA is the consequence of the aging process, manipulating the epigenetic clock could be an anti-aging

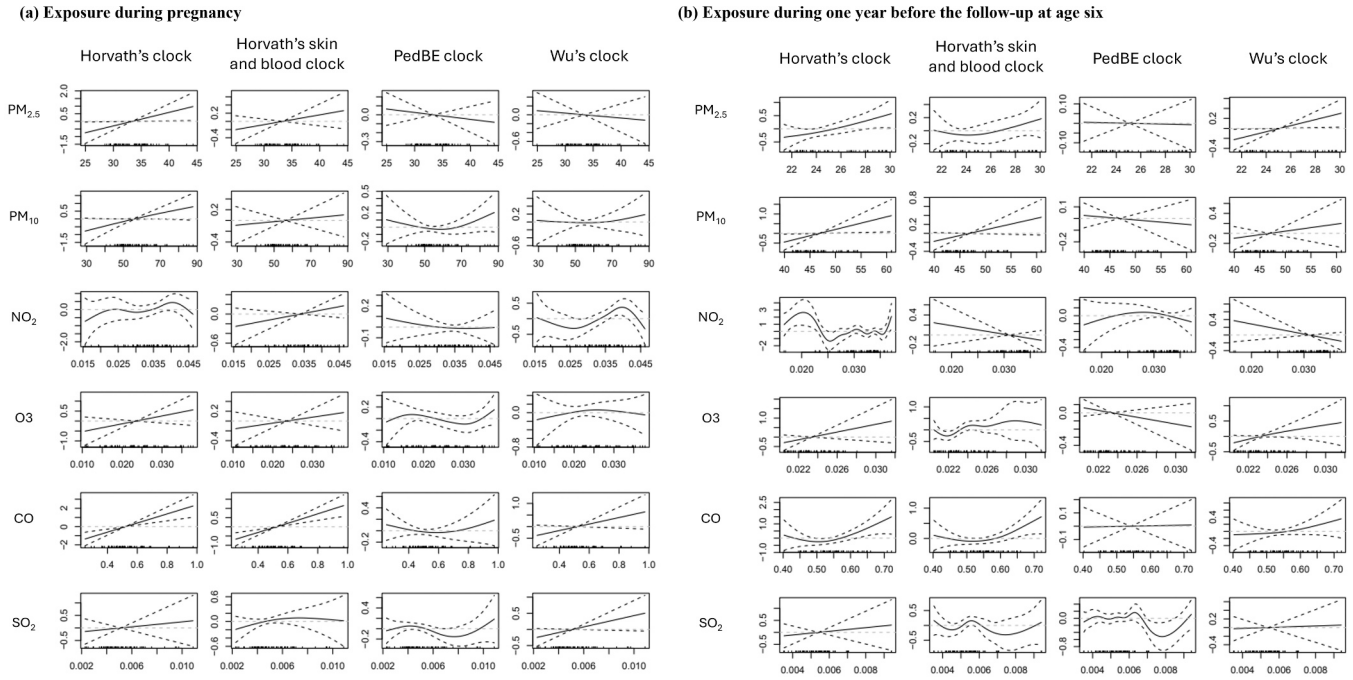


**Fig. 1.** Nonlinear associations between air pollutants and EAA.

target. Fitzgerald et al. examined the effects of a diet and lifestyle intervention on epigenetic age among older adults aged in 50–72 years, and showed the significant rejuvenation of epigenetic aging by modified lifestyle (Fitzgerald et al., 2021). On the other hand, epigenetic clocks might result from aging rather than be a cause. Horvath et al. proposed that the DNA methylation clock shows the activity of an epigenetic maintenance system and the function of maintaining epigenetic stability (Horvath, 2013b). For example, chemical agents that interfere with the maintenance and repair function of genes, such as benzene and trichloroethylene, have been reported to be closely associated with EAA (Van der Laan et al., 2022). Hence, EAA could be simply a surrogate indicator of accelerated aging caused by disturbances of decreased maintenance function of the genome.

### 4.5. Strengths and limitations

To the best of the authors' knowledge, this study is the first to examine the association between air pollution and epigenetic aging in children. A prospective mother–child cohort was used to investigate the association between air pollutant exposure and epigenetic aging, with the validated environmental exposure data, epigenetic methylation measurements, and methylation clocks. Nevertheless, this study had several limitations. First, the sample size for DNA methylation analysis was relatively small, which may affect the statistical power and generalizability of the findings. Second, assessing the epigenetic age at a single time point does not capture the dynamic nature of the epigenetic changes or the cumulative impact of air pollutant exposure. Third, potential inaccuracies in $PM_{2.5}$ modeling could introduce measurement errors, even though the random measurement error probably did not affect the direction of the observed association. Fourth, the study findings, derived from South Korean children, may have limited applicability to populations in different regions with varying environmental and genetic backgrounds. Despite these limitations, this research provides valuable insights into the effects of air pollution on epigenetic aging in children, highlighting the need for further studies with larger and more diverse populations, as well as investigations into the biological pathways. Furthermore, potential protective factors against EAA for children, such as Fahy et al., should be investigated in future studies (Fahy et al., 2019).

### 4.6. Conclusion

A significant association was found between prenatal and childhood exposure to ambient air pollutants and acceleration in epigenetic age among children. These results suggest that air pollution is significantly associated with EAA and possibly related to the potential disease status in later life.

## CRediT authorship contribution statement

**Johanna Inhyang Kim:** Writing – review & editing, Data curation. **Yun-Chul Hong:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Young Ah Lee:** Writing – review & editing, Data curation. **Bung-Nyun Kim:** Writing – review & editing, Data curation. **Soon-Tae Kim:** Writing – review & editing, Data curation. **Choong Ho Shin:** Writing – review & editing, Data curation. **Youn-Hee Lim:** Writing – review & editing, Data curation. **Yoon-Jung Choi:** Writing – review & editing, Investigation, Data curation. **Dong-Wook Lee:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization.

## 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.

## Data availability

The authors do not have permission to share data.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116823.

## References


Aryee, M.J., et al., 2014. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 30, 1363–1369.

Bock, C., et al., 2012. DNA methylation dynamics during in vivo differentiation of blood and skin stem cells. Mol. Cell 47, 633–647.

Brauer, M., et al., 2002. Air pollution from traffic and the development of respiratory infections and asthmatic and allergic symptoms in children. Am. J. Respir. Crit. care Med. 166, 1092–1098.

Breton, C.V., et al., 2016. Prenatal air pollution exposures, DNA methyl transferase genotypes, and associations with newborn LINE1 and Alu methylation and childhood blood pressure and carotid intima-media thickness in the children's health study. Environ. Health Perspect. 124, 1905–1912.

Choi, Y.-J., et al., 2023. DNA methylation is associated with prenatal exposure to sulfur dioxide and childhood attention-deficit hyperactivity disorder symptoms. Sci. Rep. 13, 3501.

De Kok, T.M., et al., 2006. Toxicological assessment of ambient and traffic-related particulate matter: a review of recent studies. Mutat. Res. /Rev. Mutat. Res. 613, 103–122.

Fahy, G.M., et al., 2019. Reversal of epigenetic aging and immunosenescent trends in humans. Aging Cell 18, e13028.

Field, A.E., et al., 2018. DNA methylation clocks in aging: categories, causes, and consequences. Mol. Cell 71, 882–895.

Fitzgerald, K.N., et al., 2021. Potential reversal of epigenetic age using a diet and lifestyle intervention: a pilot randomized clinical trial. Aging (Albany NY) 13, 9419.

Fransquet, P.D., et al., 2019. The epigenetic clock as a predictor of disease and mortality risk: a systematic review and meta-analysis. Clin. Epigenetics 11, 1–17.

Gehring, U., et al., 2013. Air pollution exposure and lung function in children: the ESCAPE project. Environ. Health Perspect. 121, 1357–1364.

Gruzieva, O., et al., 2017. Epigenome-wide meta-analysis of methylation in children related to prenatal NO2 air pollution exposure. Environ. Health Perspect. 125, 104–110.

Han, C., et al., 2020. Long-term exposure to fine particulate matter and development of chronic obstructive pulmonary disease in the elderly. Environ. Int. 143, 105895.

Hannum, G., et al., 2013. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol. Cell 49, 359–367.

World Health Organization, 2016. Ambient air pollution: A global assessment of exposure and burden of disease.

Horvath, S., 2013a. DNA methylation age of human tissues and cell types. Genome Biol. 14, 1–20.

Horvath, S., 2013b. DNA methylation age of human tissues and cell types. Genome Biol. 14, 3156.

Horvath, S., et al., 2018. Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and ex vivo studies. Aging (Albany NY) 10, 1758.

Horvath, S., Raj, K., 2018. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat. Rev. Genet. 19, 371–384.

Houseman, E.A., et al., 2012. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinforma. 13, 1–16.

Kim, H.C., et al., 2017b. Regional contributions to particulate matter concentration in the Seoul metropolitan area, South Korea: seasonal variation and sensitivity to meteorology and emissions inventory. Atmos. Chem. Phys. 17, 10315–10332.

Kim, B.-U., et al., 2017a. Spatially and chemically resolved source apportionment analysis: case study of high particulate matter event. Atmos. Environ. 162, 55–70.

Kim, K.-N., et al., 2018. Cohort profile: the Environment and Development of Children (EDC) study: a prospective children's cohort. Int. J. Epidemiol. 47, 1049–1050f.

Levine, M.E., et al., 2018. An epigenetic biomarker of aging for lifespan and healthspan. Aging (Albany NY) 10, 573.

Lopuszanska, U., Samardakiewicz, M., 2020. The relationship between air pollution and cognitive functions in children and adolescents: a systematic review. Cogn. Behav. Neurol. 33, 157–178.

Marioni, R.E., et al., 2015. DNA methylation age of blood predicts all-cause mortality in later life. Genome Biol. 16, 1–12.

McEwen, L.M., et al., 2020. The PedBE clock accurately estimates DNA methylation age in pediatric buccal cells. Proc. Natl. Acad. Sci. USA 117, 23329–23335.

Miller, M.D., Marty, M.A., 2010. Impact of environmental chemicals on lung development. Environ. Health Perspect. 118, 1155–1164.

Min, J.L., et al., 2018. Meffil: efficient normalization and analysis of very large DNA methylation datasets. Bioinformatics 34, 3983–3989.

Musci, R.J., et al., 2023. Using epigenetic clocks to characterize biological aging in studies of children and childhood exposures: a systematic review. Prev. Sci. 24, 1398–1423.

Nwanaji-Enwerem, J.C., et al., 2016. Long-term ambient particle exposures and blood DNA methylation age: findings from the VA normative aging study. Environ. Epigenetics 2, dvw006.

Patel, M.M., et al., 2013. Traffic-related air pollutants and exhaled markers of airway inflammation and oxidative stress in New York City adolescents. Environ. Res. 121, 71–78.

Pelegí-Sisó, D., et al., 2021. methylclock: a Bioconductor package to estimate DNA methylation age. Bioinformatics 37, 1759–1760.

de Prado-Bert, P., et al., 2021. The early-life exposome and epigenetic age acceleration in children. Environ. Int. 155, 106683.

Rider, C.F., Carlsten, C., 2019. Air pollution and DNA methylation: effects of exposure in humans. Clin. Epigenetics 11, 131.

Sbihi, H., et al., 2019. Prenatal exposure to traffic-related air pollution, the gestational epigenetic clock, and risk of early-life allergic sensitization. J. Allergy Clin. Immunol. 144, 1729–1731 e5.

Shi, W., et al., 2022. Personal airborne chemical exposure and epigenetic ageing biomarkers in healthy Chinese elderly individuals: evidence from mixture approaches. Environ. Int. 170, 107614.

Skamarock, W.C., Klemp, J.B., 2008. A time-split nonhydrostatic atmospheric model for weather research and forecasting applications. J. Comput. Phys. 227, 3465–3485.

Stieb, D.M., et al., 2012. Ambient air pollution, birth weight and preterm birth: a systematic review and meta-analysis. Environ. Res. 117, 100–111.

Teschendorff, A.E., et al., 2013. A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. Bioinformatics 29, 189–196.

United States Environmental Protection Agency, 2011. US Environmental Protection Agency. Exposure Factors Handbook: 2011 Edition (Final Report). Office of Health and Environmental Assessment, US Environmental Protection.

Van der Laan, L., et al., 2022. Epigenetic aging biomarkers and occupational exposure to benzene, trichloroethylene and formaldehyde. Environ. Int. 158, 106871.

Ward-Caviness, C.K., et al., 2016. Long-term exposure to air pollution is associated with biological aging. Oncotarget 7, 74510.

White, A.J., et al., 2019. Air pollution, particulate matter composition and methylation-based biologic age. Environ. Int. 132, 105071.

Wu, X., et al., 2019. DNA methylation profile is a quantitative measure of biological aging in children. Aging (Albany NY) 11, 10031.

Xia, X., et al., 2017. Molecular and phenotypic biomarkers of aging. F1000Research 6.