Epigenome-Wide Association of Neonatal Methylation and Prenatal PM2.5 Exposure Suggests Timing-Specific Effects

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# Supplementary Materials

#### Average Prenatal PM2.5 Exposure

##### Trimester 1

Analyzing average PM2.5 concentrations over trimester 1 generated a genomic control factor of 0.941 and a QQ plot showing minimal deviation of the observed p-value distribution from the expected under the null hypothesis (eFigure 1). The SVA was not included in the model as it negatively impacted the QQ plot.

Chart, line chart

Description automatically generated

**eFigure 1.** QQ plot for the analysis between DNA methylation at each studied CpG site and PM2.5 averaged over trimester 1 of pregnancy with observed and expected p-values transformed on a negative log10 scale.

Chart, scatter chart

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**eFigure 2.** Manhattan plots for the association between DNA methylation at each studied CpG site and PM2.5 averaged over trimester 1 of pregnancy adjusted for specified covariates. The vertical axis plots the negative log10 p-value for each association. The Bonferroni cutoff for significance is denoted by the horizontal red line.

##### Trimester 2

Analyzing average PM2.5 concentrations over trimester 2 generated a genomic control factor of 1.01 and a QQ plot with minimal deviations from expected (eFigure 3). This model was additionally adjusted for SVA as its inclusion greatly improved the QQ plot and the genomic control factor.

Chart, line chart

Description automatically generated

**eFigure 3.** QQ plot for the analysis between DNA methylation at each studied CpG site and PM2.5 averaged over trimester 2 of pregnancy with observed and expected p-values transformed on a negative log10 scale.

Chart

Description automatically generated

**eFigure 4.** Manhattan plots for the association between DNA methylation at each studied CpG site and PM2.5 averaged over trimester 2 of pregnancy adjusted for specified covariates. The vertical axis plots the negative log10 p-value for each association. The Bonferroni cutoff for significance is denoted by the horizontal red line.

##### Combined

Analyzing average PM2.5 concentrations across the first two trimesters generated a genomic control factor of 0.881 and a QQ plot with minimal deviations from expected (eFigure 5). The SVA was not included in the model as it negatively impacted the QQ plot.

Chart, line chart

Description automatically generated

**eFigure 5.** QQ plot for the analysis between DNA methylation at each studied CpG site and PM2.5 averaged over trimesters 1 and 2 of pregnancy with observed and expected p-values transformed on a negative log10 scale.