Supplemental Material

Prenatal Exposure to Mercury: Associations with Global DNA methylation and Hydroxymethylation in Cord Blood and in Childhood

Andres Cardenas, Sheryl L. Rifas-Shiman, Lode Godderis, Radu-Corneliu Duca, Ana Navas-Acien, Augusto A. Litonjua, Dawn L. DeMeeo, Kasey J. Brennan, Chitra J. Amarasiriwardena, Marie-France Hivert, Mathew W. Gillman, Emily Oken, and Andrea A. Baccarelli

Table of Contents

Table S1. Multivariate linear models for the associations of estimated cell type composition in cord blood with 5-hydroxymethylcytosine content (%-5hmC), 5-methylcytosine content (%-5mC) and their ratio (N=473).

Table S2. a Adjusted association between global measures of DNA methylation (%-5mC) and DNA hydroxymethylation (%-5hmC) in cord blood and early childhood blood samples with cognitive function assessed during early childhood.

Figure S1. Diagram of the sample flow for the study: repeated measurements across different time-points for all epigenomic measurements and for the epigenomic measurements restricted to complete covariate and exposure information.

Figure S2. Bean plots of the distributions of 5-hydroxymethylcytosine (5hmC) and 5-methylcytosine (5mC) in cord blood, early childhood and mid-childhood blood (black lines represent the mean levels of 5hmC and 5mC at sample collection and dotted line represents overall mean).

Figure S3. a Fully adjusted association for A) %-5hmC B) %-5mC and C) %-difference in the ratio of 5mC to 5hmC per doubling in prenatal maternal mercury concentrations. Estimates for cord blood were restricted to either early (■) or mid-childhood (▲) samples for sensitivity analyses.

Figure S4. Spearman correlations coefficients for CpGs previously associated with prenatal maternal mercury exposure within this cohort and global measures of DNA methylation (%-5mC) and DNA hydroxymethylation (%-5hmC). Color saturation indicates strength of spearman correlation coefficient, white indicates non-significant correlations P>0.05.
Figure S5. Adjusted EWAS of %-5mC and DNA methylation from the 450K in cord blood: (A) QQ-plot and genomic inflation (B) volcano-plot and multiple comparison adjustment and (C) volcano-plot for the association of prenatal maternal RBC-Hg and 2,415 CpGs significantly associated with %-5hmC (No CpG passed the FDR<0.05 for the 2,415 tests performed in C).

Figure S6. Scatter plots and spearman correlations for the association of LINE-1 DNA methylation in cord blood and (A) %-5hmC in cord blood (B) %-5mC in cord blood and (C) prenatal maternal RBC-Hg concentration (red: fitted regression line). P>0.30 for all correlations.

Supplementary Methods.

Table S3. MS/MS parameters for specific detection by MRM for each target compound.

Figure S7. Calibration curves for (A) Cytosine [C], (B) 5-methylcytosine [5mC], and (C) 5-hydroxymethylcytosine [5hmC].

Table S4. Parameters of validation of the UPLC-MS/MS method for the determination of global DNA methylation and hydroxymethylation.

References.