

Supplementary Materials: Early Pregnancy Exposure to Ambient Air Pollution among Late-Onset Preeclamptic Cases is Associated with Placental DNA Hypomethylation of Specific Genes and Slower Placental Maturation

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Table S1. Differences in gestational age between combined PE status and NO_x exposure groups, analysed using linear regression.

PE status and NO _x group	beta ^a	Std error	P-value
1. Controls, low NO _x	reference	reference	reference
2. Controls, high NO _x	-0.14	0.23	0.53
3. Cases, low NO _x	-0.28	0.36	0.44
4. Cases, high NO _x	-0.61	0.28	0.035*
Model p-value			0.20

^aBeta is derived from the following linear model: Epigenetic age = intercept + β_1 * PE status and NO_x group combination (categorical).

Table S2. Differentially methylated positions (DMPs) when comparing controls with high NO_x exposure, (reference) vs. PE cases with high NO_x exposure in pregnancies with female fetuses. The twelve DMPs with lowest q-value are already presented in Table 2 in the main text, thus the DMPs presented here are the DMPs ordered 14–50 ranked by q-value.

Cpg	Chr	Gene	Gene name	2 ^{logFC} (95% CI) ^a	Beta ^b	p-value	q-value
cg15812969	21	USP25	ubiquitin specific peptidase 25	0.71 (0.63, 0.80)	0.41	0.000000339	0.019
cg22356173	17	CLUH	clustered mitochondria homolog	0.59 (0.49, 0.71)	0.05	0.000000350	0.019
cg10926412	13	NA ^c		0.61 (0.51, 0.72)	0.40	0.000000385	0.020
cg25030347	17	RPTOR	regulatory associated protein of MTOR complex 1	0.43 (0.32, 0.57)	0.88	0.000000448	0.022
cg06343806	7	NA		0.62 (0.52, 0.73)	0.36	0.000000541	0.022
cg17936567	22	TAB1	TGF-beta activated kinase 1 (MAP3K7) binding protein 1	0.74 (0.67, 0.82)	0.28	0.000000549	0.022

cg13923903	22	ZNRF3	zinc and ring finger 3	0.70 (0.61, 0.79)	0.40	0.000000587	0.022
cg25141112	8	LINC01298	long intergenic non-protein coding RNA 1298(LINC01298)	0.71 (0.64, 0.80)	0.61	0.000000615	0.022
cg26187194	10	NA		2.91 (2.00, 4.23)	0.70	0.000000623	0.022
cg10590292	12	BIN2	bridging integrator 2	0.44 (0.33, 0.59)	0.68	0.000000626	0.022
cg23745445	15	ANP32A	acidic nuclear phosphoprotein 32 family member A	0.65 (0.56, 0.76)	0.23	0.000000628	0.022
cg18686217	3	DAG1	dystroglycan 1	0.67 (0.58, 0.77)	0.61	0.000000829	0.024
cg18014788	18	PTPRM	protein tyrosine phosphatase, receptor type M	0.52 (0.41, 0.65)	0.84	0.000000869	0.024
cg01234517	7	ACTR3C	ARP3 actin-related protein 3 homolog C	0.48 (0.37, 0.63)	0.79	0.000000930	0.024
cg08304968	20	NA		0.66 (0.56, 0.76)	0.53	0.000000945	0.024
cg04728805	5	FAM53C	family with sequence similarity 53 member C	0.64 (0.55, 0.75)	0.10	0.000000976	0.024
cg16849481	11	SPDYC	speedy/RINGO cell cycle regulator family member C	0.69 (0.61, 0.79)	0.39	0.000000995	0.024
cg06154002	14	TDRD9	tudor domain containing 9	2.47 (1.79, 3.41)	0.33	0.000001008	0.024
cg16414660	2	DGKD	diacylglycerol kinase delta(DGKD)	0.59 (0.49, 0.72)	0.70	0.000001046	0.024
cg01584478	4	LRBA	LPS responsive beige-like anchor protein	0.62 (0.52, 0.73)	0.43	0.000001080	0.024
cg11548591	9	DAB2IP	DAB2 interacting protein	0.54 (0.43, 0.67)	0.67	0.000001112	0.024
cg23502295	6	MICAL1	microtubule associated monooxygenase, calponin and LIM domain containing 1	0.68 (0.59, 0.78)	0.81	0.000001116	0.024
cg16013484	5	NA		0.59 (0.48, 0.71)	0.72	0.000001164	0.024
cg17971961	1	KIAA0319L	KIAA0319 like	0.67 (0.59, 0.78)	0.69	0.000001169	0.024
cg07249517	3	MB21D2	Mab-21 domain containing 2	0.70 (0.62, 0.80)	0.55	0.000001188	0.024
cg02712011	3			0.65 (0.56, 0.76)	0.11	0.000001233	0.024
cg25473467	6	ANKRD6	ankyrin repeat domain 6	0.62 (0.53, 0.74)	0.12	0.000001254	0.024
cg25569590	7	COBL	cordon-bleu WH2 repeat protein	0.55 (0.44, 0.68)	0.24	0.000001265	0.024
cg19581707	22	SLC25A18	solute carrier family 25 member 18	0.68 (0.59, 0.78)	0.30	0.000001281	0.024

cg22490758	11	PC	pyruvate carboxylase	0.71 (0.63, 0.81)	0.71	0.000001302	0.024
cg07326313	15	NA		0.56 (0.45, 0.69)	0.30	0.000001316	0.024
cg24025567	7	CUX1	cut like homeobox 1	0.68 (0.60, 0.79)	0.56	0.000001344	0.024
cg11071407	6	PPARD	peroxisome proliferator activated receptor delta	0.61 (0.50, 0.73)	0.61	0.000001397	0.024
cg05844827	17	MSI2	musashi RNA binding protein 2	0.62 (0.53, 0.74)	0.45	0.000001432	0.024
cg00821201	20	DSTN	destrin, actin depolymerizing factor	0.63 (0.53, 0.75)	0.51	0.000001509	0.024
cg12906748	11	C11orf16	chromosome 11 open reading frame 16	0.63 (0.54, 0.75)	0.56	0.000001512	0.024
cg25688280	6	RANBP9	RAN binding protein 9	0.69 (0.60, 0.79)	0.39	0.000001514	0.024

Abbreviations: Chr, chromosome; CI, confidence interval; q -value, False discovery rate (FDR)-adjusted p -value using the Benjamini-Hochberg method; FC, fold change. ^a $2^{\log FC}$, binary logarithmic fold change. LogFC denotes β_i from the following robust regression model: M-value = $\beta_1 \times \text{PE/NO}_x \text{ group} + \beta_2 \times \text{DNA concentration} + \beta_3 \times \text{gestational age} + \beta_4 \times \text{estimated fraction Hofbauer cells} + \beta_5 \times \text{estimated fraction Syncytiotrophoblast cells}$. ^bAverage methylation state, expressed as Beta-value, for all study participants, ranging from 0 to 1 (1 means fully methylated). ^cNA, not annotated, i.e., the CpG is not present in any known gene.

Table S3. Top Gene Ontology (GO) terms for analyses including genes with CpGs with $q < 0.1$ in the DMP analyses for different combined PE status and NO_x group comparisons.

GO ID	Term	Total Nr of genes in Term	Nr of genes from DMPs with $q < 0.1$	unadjusted p -value	q -value
<i>Controls with low NO_x exposure(reference) vs. PE cases and high NO_x exposure</i>					
GO:0072080	nephron tubule development	88	5	0.000027	0.30
GO:0061326	renal tubule development	90	5	0.000031	0.30
GO:0050508	glucuronosyl-N-acetylglucosaminyl-proteoglycan 4-alpha-N-acetylglucosaminyltransferase activity	3	2	0.000049	0.30
GO:0072009	nephron epithelium development	104	5	0.000053	0.30
GO:0009595	detection of biotic stimulus	29	3	0.000081	0.37
GO:0072006	nephron development	137	5	0.000187	0.56
GO:0072088	nephron epithelium morphogenesis	72	4	0.000198	0.56
GO:0072073	kidney epithelium development	135	5	0.000203	0.56
GO:0072028	nephron morphogenesis	74	4	0.000222	0.56
GO:0032490	detection of molecule of bacterial origin	11	2	0.000371	0.71
GO:0072257	metanephric nephron tubule epithelial cell differentiation	7	2	0.000402	0.71
GO:0072307	regulation of metanephric nephron tubule epithelial cell differentiation	7	2	0.000402	0.71
<i>Controls with high NO_x exposure (reference) vs. PE cases with high NO_x exposure</i>					
GO:0032570	response to progesterone	44	4	0.000270	1
GO:0051016	barbed-end actin filament capping	20	3	0.000544	1
GO:0005996	monosaccharide metabolic process	270	8	0.000620	1
GO:2000813	negative regulation of barbed-end actin filament capping	4	2	0.000668	1
GO:0009595	detection of biotic stimulus	29	3	0.000729	1
GO:2000812	regulation of barbed-end actin filament capping	5	2	0.000790	1
GO:0001820	serotonin secretion	9	2	0.000875	1
GO:0016861	intramolecular oxidoreductase activity, interconverting aldoses and ketoses	8	2	0.000988	1
GO:0032490	detection of molecule of bacterial origin	11	2	0.001350	1
GO:0019318	hexose metabolic process	230	7	0.001428	1
<i>Female pregnancies only - Controls with high NO_x exposure (reference) vs. PE cases with high NO_x exposure</i>					
GO:0099186	structural constituent of postsynapse	10	6	0.000003	0.06
GO:0098918	structural constituent of synapse	16	7	0.000013	0.15
GO:0008089	anterograde axonal transport	48	9	0.000106	0.66
GO:0036464	cytoplasmic ribonucleoprotein granule	209	19.5	0.000117	0.66
GO:0035770	ribonucleoprotein granule	220	19.5	0.000218	0.97
GO:0030705	cytoskeleton-dependent intracellular transport	178	18	0.000274	0.97
GO:0035033	histone deacetylase regulator activity	4	3	0.000332	0.97
GO:0003727	single-stranded RNA binding	93	11	0.000343	0.97
GO:0007006	mitochondrial membrane organization	130	13	0.000485	1
GO:1904115	axon cytoplasm	57	9	0.000503	1

Table S4. Comparisons between the study populations included in the placental DNA methylation analyses and RNA sequencing analyses ^a.

Characteristics	DNA methylation (n = 111)	RNA sequencing (n = 17)
Preeclampsia	29 (26.1)	10 (58.9)
Maternal age		
<35	90 (81.1)	14 (82.4)
35≤	21 (18.9)	3 (17.6)
Maternal pregestational BMI		
<18.5	1 (0.9)	0
18.5-24.9	55 (49.5)	7 (41.2)
25-29.9	32 (28.8)	2 (11.8)
30≤	23 (20.7)	8 (47.0)
Maternal Parity		
Nullipara	77 (69.4)	10 (58.8)
Primipara	26 (23.4)	4 (23.5)
Multipara	8 (7.2)	3 (17.6)
Previous Gestational Hypertension	2 (1.8)	0
Mode of delivery		
Vaginal	89 (80.2)	16 (92.3)
Cesarean	22 (19.8)	1 (5.9)
Fetal sex		
Male	59 (53.2)	8 (47.0)
Female	52 (46.8)	9 (53.0)
Gestational age by days	274.5±12.8	277 ± 7.8
Preterm birth	8 (7.2)	0
Fetal weight (g)	3432±621	3655±383
Year of birth		
2008	21 (18.9)	5 (29.4)
2009	29 (26.1)	4 (23.5)
2010	2 (1.8)	1 (5.9)
2011	21 (18.9)	4 (29.4)
2014	18 (16.2)	1 (5.9)
2015	20 (18.0)	2 (11.8)

^a Mean, SD are presented for continuous variables, and numbers (percentages) are presented for categorical variables.

A)

↓ PE status NO _x exposure →	Low	High
Control	Group 1 N = 45	Group 2 N = 37
Case	Group 3 N = 10	Group 4 N = 19

B)

Pair-wise group comparisons	
Controls, high NO _x	VS. Controls, low No _x
PE cases, low NO _x	VS. Controls, low No _x
PE cases, high NO_x	VS. Controls, low No_x
PE cases, low NO_x	VS. Controls, high No_x
PE cases, high NO_x	VS. Controls, high No_x
PE cases, high NO _x	VS. PE cases, low NO _x
PE cases, low NO _x	VS. Controls, low No _x

Figure S1. Summary of groups based on a combination of pregnancy status (PE or normotensive control) and exposure to ambient NO_x (low or high NO_x exposure based on median split) (Table A) and the pair-wise comparisons performed in the DMP analyses, where comparisons that had DMPs ($q < 0.05$) are marked in bold (Table B).

