

Supplemental Material

Prenatal Exposure to Maternal Cigarette Smoking and DNA Methylation: Epigenome-Wide Association in a Discovery Sample of Adolescents and Replication in an Independent Cohort at Birth through 17 Years of Age

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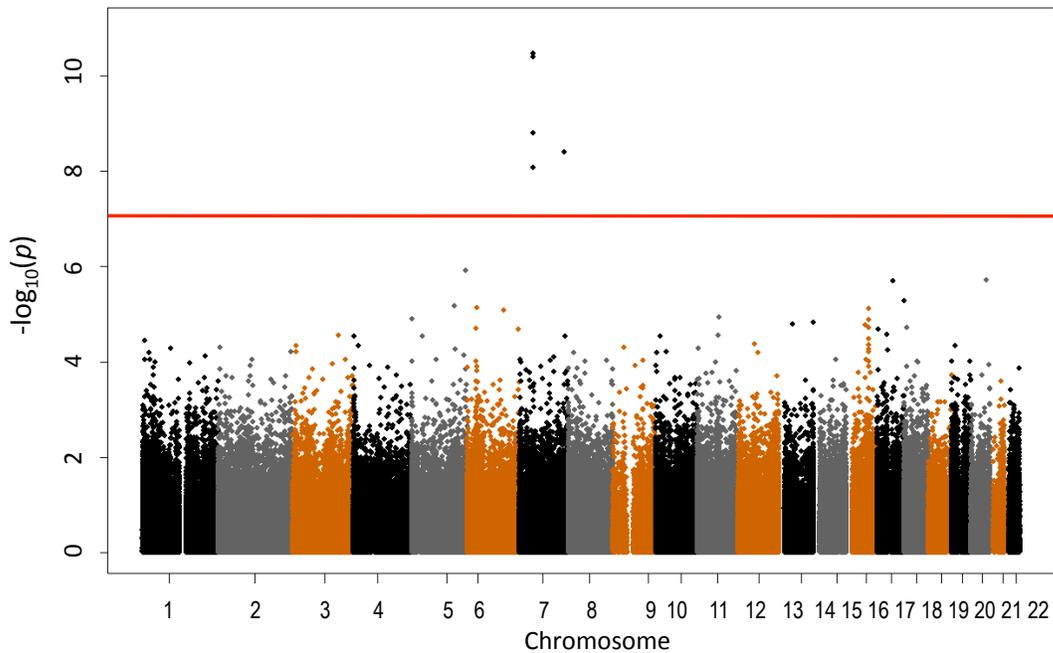
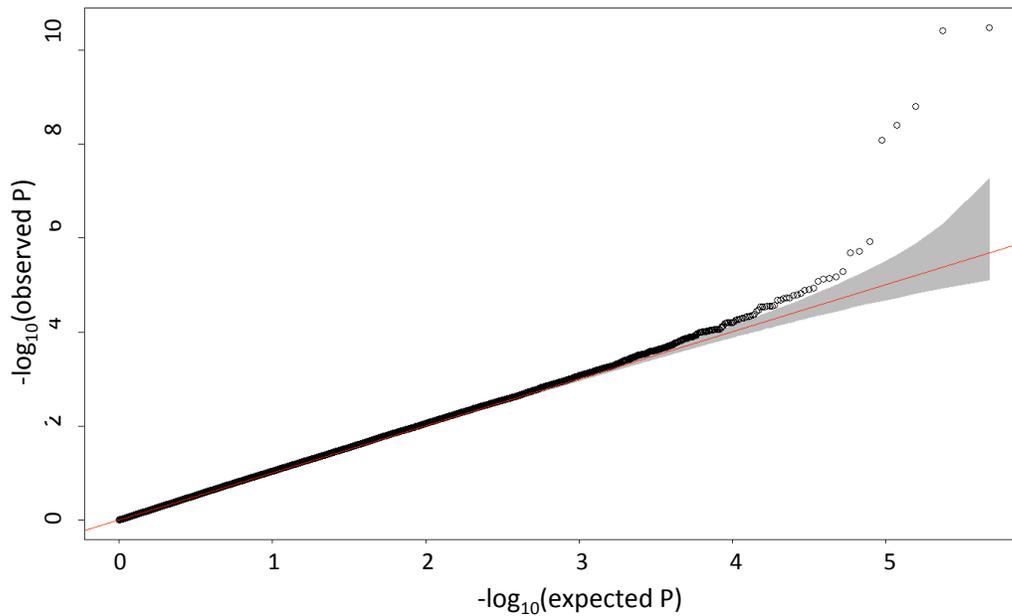
A)**B)**

Figure S1. EWAS in the discovery SYS sample – Manhattan and quantile-quantile plots. (A) Manhattan plot for Model A (adjusted for age, sex, batch and blood cell fractions) is shown. Horizontal line indicates Bonferroni-corrected statistical significance at $p < 0.05$ (uncorrected $p = 1.1 \times 10^{-7}$). (B) Quantile-quantile plot for Model A (adjusted for age, sex, batch and blood cell fractions) is presented. P-values for observed vs. expected association strength are shown. The $y=x$ line is indicated as solid diagonal line.

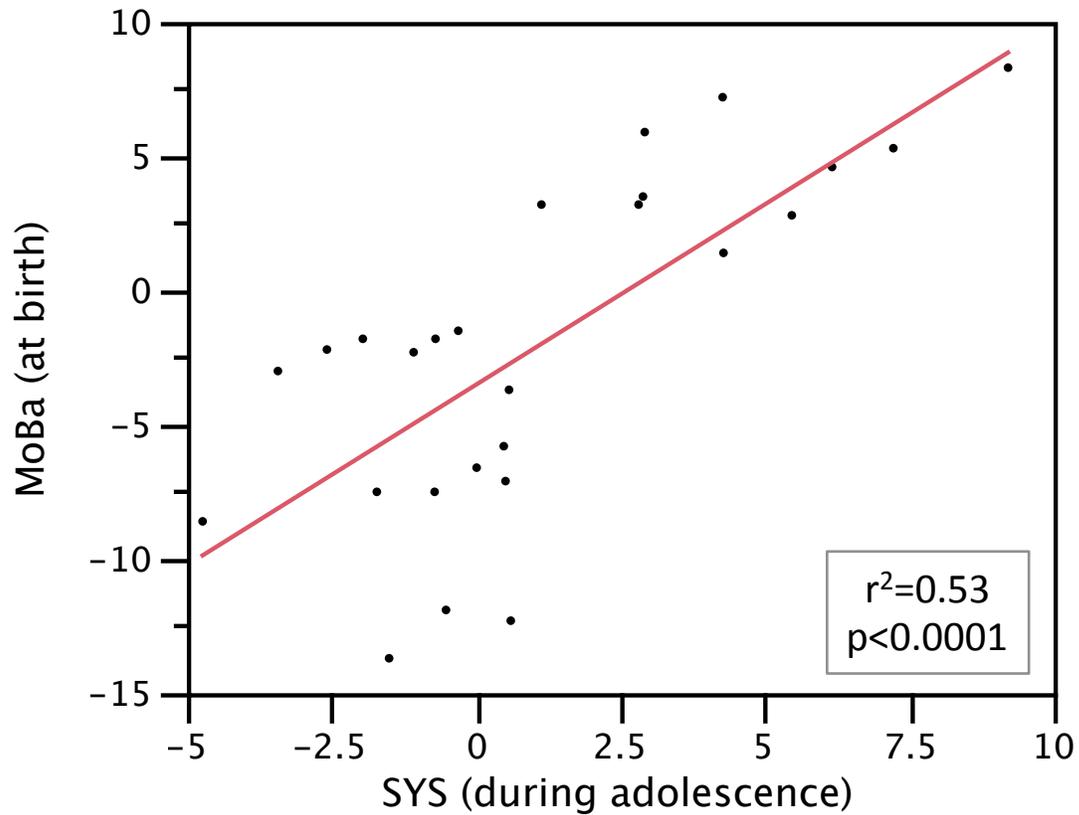


Figure S2. EWAS-significant CpGs from the MoBa sample of newborns* in the discovery SYS sample of adolescents. Adjusted differences in DNAm β values between exposed and non-exposed individuals (DNAm β differences) are plotted. Pearson correlation test was performed.

*Joubert, B.R. et al. (2012) *Environ Health Perspect*, 120, 1425-1431.

Table S1. Basic characteristics of ALSPAC participants at birth (n = 441) and ages 7 (n = 447) and 17 years (n = 219) (replication cohort).

Characteristic	At birth NE	At birth E	P-value	Age 7 NE	Age 7 E	P-value	Age 17 NE	Age 17 E	P-value
No.	402	39		408	39		206	13	
Sex (males/females)	210/192	17/22	0.3	206/202	17/22	0.41	85/121	5/8	0.84
Age (years)	0	0	N/A	7.5 ± 0.12	7.5 ± 0.20	0.21	17.7 ± 0.3	17.6 ± 0.6	0.95
Maternal education (proportions, %)*	14/34/33/19	31/49/15/5	0.001	13/34/35/18	31/49/18/2	<0.001	12/33/37/18	23/46/31/0	0.22
Parental social class (proportions, %)**	21/43/27/5/4/0	3/28/20/36/10/3	<0.001	22/42/27/6/3/0	2/24/23/33/15/3	<0.001	26/41/25/6/2/0	7/31/23/23/8/8	<0.001
Maternal cigarette smoking (1 st trimester, # cigarettes/day)	0	13.8 ± 6.0	N/A	0	13.3 ± 6.1	N/A	0	13.9 ± 6.0	N/A
Maternal cigarette smoking (2 nd trimester, # cigarettes/day)	0	12.8 ± 5.1	N/A	0	12.5 ± 5.2	N/A	0	12.4 ± 5.9	N/A
Maternal cigarette smoking (3 rd trimester, # cigarettes/day)	0	12.3 ± 5.5	N/A	0	12.2 ± 5.6	N/A	0	13.0 ± 6.4	N/A
Maternal age at delivery (years)	30.0 ± 4.2	27.7 ± 5.7	0.004	29.9 ± 4.3	27.4 ± 5.8	0.002	30.1 ± 4.4	27.8 ± 5.3	0.07
Length of gestation (weeks)	39.6 ± 1.5	38.9 ± 2.4	0.03	39.5 ± 1.5	38.9 ± 2.4	0.02	39.5 ± 1.4	38.7 ± 2.9	0.06
Birth weight (g)	3488 ± 482	3139 ± 490	<0.001	3500 ± 487	3171 ± 496	<0.001	3432 ± 441	3090 ± 521	0.008
Participant smoking (proportions, %)	N/A	N/A	N/A	N/A	N/A	N/A	12	50	0.003
Postnatal parent smoking (proportions, %)	N/A	N/A	N/A	11.1	89	<0.001	16	75	<0.001

Data are presented as mean ± standard deviation. Exposed (E) and non-exposed (NE) indicates individuals who were and were not, respectively, exposed prenatally to maternal cigarette smoking.

*Maternal education was classified as the highest achieved level: (i) vocational/CSE, (ii) O-level (passed examinations at age 16), (iii) A-level (passed examinations at age 18), or (iv) degree.

**Parental social class was classified as follows: i) professional, ii) managerial or technical, iii-a) skilled non-manual, iii-b) skilled manual, iv) partly skilled manual, or v) unskilled manual.

Table S2. EWAS in the discovery sample of SYS adolescents (not adjusted for any potential confounders, all participants included [66 exposed vs. 66 non-exposed]).

CpG ID	Position	Gene	DNAm β difference (SE)	P-value
cg12803068	7: 44969444	<i>MYO1G</i>	0.09 (0.01)	1.25E-11*
cg04180046	7: 44969261	<i>MYO1G</i>	0.07 (0.01)	5.06E-11*
cg22132788	7: 44969011	<i>MYO1G</i>	0.05 (0.01)	8.99E-10*
cg25949550	7: 145445239	<i>CNTNAP2</i>	-0.02 (0.00)	1.65E-09*
cg19089201	7: 44968812	<i>MYO1G</i>	0.04 (0.01)	8.78E-09*
cg25583601	5: 33476393	<i>TARS</i>	-0.01 (0.00)	1.35E-06
cg17924476	5: 376794	<i>AHRR</i>	0.03 (0.01)	1.54E-06
cg23916896	5: 421804	<i>AHRR</i>	-0.04 (0.01)	1.88E-06
cg15982595	13: 112349181	<i>C13orf35</i>	-0.01 (0.00)	2.91E-06
cg00253658	16: 52767997	NA	0.07 (0.01)	4.48E-06
cg14179389	1: 92720549	<i>GFI1</i>	-0.05 (0.01)	6.17E-06
cg11902777	5: 421843	<i>AHRR</i>	-0.02 (0.00)	6.91E-06
cg15507334	10: 14412919	<i>FRMD4A</i>	0.03 (0.01)	7.81E-06
cg23986376	6: 28935012	NA	-0.02 (0.00)	1.02E-05
cg04118462	3: 151012838	<i>RNF1</i>	-0.02 (0.00)	1.14E-05
cg21330896	8: 28261624	<i>ZNF395</i>	-0.04 (0.01)	1.45E-05
cg03521044	7: 20805089	NA	-0.01 (0.00)	1.45E-05
cg10805956	1: 154964248	<i>ISG20L2; C1orf66</i>	-0.02 (0.00)	2.03E-05
cg27214774	20: 48687117	<i>FAM65C</i>	0.01 (0.00)	2.30E-05
cg06841024	3: 12908799	NA	-0.01 (0.00)	2.65E-05
cg20232325	12: 129889375	<i>STX2</i>	-0.01 (0.00)	3.02E-05
cg00729656	7: 98390638	<i>TRRAP</i>	-0.02 (0.00)	3.18E-05
cg12050358	17: 33866435	<i>ARHGAP23</i>	-0.04 (0.01)	3.18E-05
cg07576541	1: 151897507	<i>SNAPIN</i>	-0.02 (0.00)	3.22E-05
cg12182991	3: 188939158	<i>BCL6</i>	-0.01 (0.00)	4.10E-05

Differential DNA methylation (DNAm) between adolescents exposed and non-exposed prenatally to maternal cigarette smoking was assessed with linear regression using M-values at 473,395 CpGs. The results are presented as adjusted mean differences in DNAm β values between exposed and non-exposed individuals (DNAm β difference) and their respective standard errors (SEs) and uncorrected (nominal) P-values. * Indicates FDR-significance ($p < 0.05$). Chromosome position (indicated by chromosome number followed by position) is based on the NCBI Human Reference Genome Assembly Build 37.p10.

References

Joubert BR, Håberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK, Huang Z, Hoyo C, Middtun Ø, Cupul-Uicab LA, Ueland PM, Wu MC, Nystad W, Bell DA, Peddada SD, London SJ. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ Health Perspect.* 2012; 120: 1425-31.