

Supplementary Material

Tables S1A-S1C. Associations between maternal metabolic parameters and other sample characteristics in PlanS cohort.

Table S1A. Association between maternal anthropometric parameters and continuous sample characteristics.

	pBMI		GWG	
	r_s	p -value	r_s	p -value
GWG	-0.20	0.004	-0.20	0.004
Birth weight	0.27	0.0001	0.16	0.024
Ponderal index	0.22	0.002	0.10	0.173
Maternal age	0.04	0.578	0.003	0.963
Gestational age	-0.05	0.506	0.08	0.286

Statistically significant findings are shown in bold. GWG, gestational weight gain; pBMI, pre-gestational body mass index; r_s , Spearman's correlation coefficient.

Table S1B. Association between maternal anthropometric parameters and categorical sample characteristics.

	N	pBMI (kg/m ²)	GWG (kg)
Parity			
primipara	72	22.6 (20.9–26.2)	14 (11–17)
multipara	131	24.6 (21.6–29.4)	13 (10–16)
p -value		0.017	0.162
Glucose tolerance status			
normal glucose tolerance	104	22.6 (20.8–26.0)	14 (11–18)
gestational diabetes mellitus	99	25.9 (22.2–31.4)	12 (8–15)
p -value		<0.0001	0.0001
Newborn sex			
female	98	23.2 (21.3–26.9)	13 (10–16)
male	105	24.1 (21.6–29.1)	13 (10–17)
p -value		0.255	0.550
Smoking in pregnancy			
no	135	23.1 (21.3–29.1)	13 (10–16)
yes	57	23.4 (21.6–27.6)	14 (11–17)
p -value		0.993	0.100

Data are reported as median [interquartile range]. p -values for the difference between the groups were determined using Mann-Whitney test. Statistically significant differences are in bold. GWG, gestational weight gain; pBMI, pre-gestational body mass index.

18 **Table S1C.** Association between maternal glucose tolerance status and other sample characteristics.

	NGT (N = 104)	GDM (N = 99)	<i>p</i> -value
Maternal BMI before pregnancy, kg/m ²	22.6 [20.8-26.0]	25.9 [22.2-31.4]	<0.0001 ^b
Weight gain during pregnancy, kg	14.3 [11.0-18.0]	12.0 [8.0-15.0]	0.0001 ^b
Maternal age at childbirth, years	33.2 [29.7-36.6]	34.2 [31.4-38.0]	0.023 ^c
Gestational age at childbirth, weeks	39.3 [38.8-39.7]	39.1 [38.6-39.6]	0.141 ^c
Newborn birth weight, g	3425 [3193-3810]	3500 [3180-3820]	0.322 ^c
Newborn ponderal index, g/cm ³	2,81 [2,70-2,97]	2,81 [2,66-3,02]	0.393 ^d
Parity (primipara/multipara), N (%)	38 (36.5)/66 (63.5)	34 (34.3)/65(65.7)	0.771 ^d
Newborn sex (female/male), N (%)	51 (49.0)/53 (51.0)	47 (47.5)/52 (52.5)	0.889 ^d
Tobacco use in pregnancy (no/yes), N (%) ^a	71 (71.7)/28 (28.3)	64 (68.8)/29 (31.2)	0.752 ^d

19 NGT, normal glucose tolerance; GDM, gestational diabetes mellitus; BMI, body mass index.
20 Continuous data are reported as median [interquartile range]. Categorical data are reported as number of subjects (N) and
21 percentage (%).

22 ^a Women who reported never having smoked or having quit smoking at least 6 months before pregnancy were categorized as
23 non-smokers, while women who reported having smoked throughout pregnancy or having quit smoking during pregnancy
24 were categorized as smokers; unclear cases (5 NGT, 6 GDM) were treated as missing data.

25 *p*-values for the difference between the NGT and GDM groups were determined using ^bMann-Whitney test, ^cStudent's t-
26 test, or ^dFisher's exact test. Statistically significant differences are in bold.
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28 **Table S2.** CpG sites targeted by methylation analyses in PlaNS cohort.

Gene	CpG site #	Chr	GRCh38 coordinates ^a	RefSeq coordinates ^b	Location in gene	TSS (bp) postition ^c	Illumina ID ^d
<i>SLC6A4</i>	1	17	Chr17: 30 235 532	5405	Intron 1	-13573	/
	2	17	Chr17: 30 235 527	5410	Intron 1	-13568	/
	3	17	Chr17: 30 235 519	5418	Intron 1	-13560	/
	4	17	Chr17: 30 235 512	5425	Intron 1	-13553	/
	5	17	Chr17: 30 235 504	5433	Intron 1	-13545	/
	6	17	Chr17: 30 235 490	5447	Intron 1	-13531	/
	7	17	Chr17: 30 235 482	5455	Intron 1	-13523	/
	8	17	Chr17: 30 235 475	5462	Intron 1	-13516	/
	9	17	Chr17: 30 235 472	5465	Intron 1	-13513	/
	10	17	Chr17: 30 235 457	5480	Intron 1	-13498	cg03363743
	11	17	Chr17: 30 235 448	5489	Intron 1	-13489	/
	12	17	Chr17: 30 235 272	5665	Intron 1	-13313	/
	13	17	Chr17: 30 235 247	5690	Intron 1	-13288	/
	14	17	Chr17: 30 235 203	5734	Intron 1	-13244	cg22584138
	15	17	Chr17: 30 235 201	5736	Intron 1	-13242	/
<i>MAOA</i>	1	X	Chr23: 43 656 361	5201	Exon 1	18	/
	2	X	Chr23: 43 656 368	5208	Exon 1	25	/
	3	X	Chr23: 43 656 370	5210	Exon 1	27	/
	4	X	Chr23: 43 656 383	5223	Exon 1	40	/
	5	X	Chr23: 43 656 386	5226	Exon 1	43	/
	6	X	Chr23: 43 656 392	5232	Exon 1	49	/
	7	X	Chr23: 43 656 398	5238	Exon 1	55	/
	8	X	Chr23: 43 656 427	5267	Intron 1	84	/
	9	X	Chr23: 43 656 432	5272	Intron 1	89	/
<i>HTR2A</i>	1	13	Chr13: 46 897 571	4464	Promoter	-1665	cg02027079
	2	13	Chr13: 46 897 130	4905	Promoter	-1224	cg27068143

29 Chr, chromosome.

30 ^a Position in Genome Reference Consortium human genome build GRCh38.p14/hg38

31 ^b Position in gene-specific reference sequence (RefSeq) NG_011747.2, NG_008957.2, and NG_013011.1 for *SLC6A4*,
32 *MAOA*, and *HTR2A*, respectively.

33 ^c Position in base pairs (bp) relative to translation start site (TSS).

34 ^d CpG locus designation in the Illumina CG database.

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36 **Table S3.** Methylation levels (%) at individual CpG sites in PlaNS participants.

Gene	CpG site #	Integral sample (N=203)	Females (N=98)	Males (N=105)	p-value
<i>SLC6A4</i>	1	3.54 [2.91-4.34]	3.87 [3.10-4.71]	85.28 [82.13-87.26]	<0.0001^a
	2	10.99 [9.65-12.32]	11.70 [10.48-13.20]	88.9 [86.82-90.41]	<0.0001^a
	3	15.28 [13.74-17.25]	15.99 [14.58-18.06]	3.25 [2.56-3.86]	<0.0001^a
	4	9.53 [8.41-10.68]	10.16 [9.24-11.20]	10.14 [8.87-11.47]	<0.0001^a
	5	15.72 [13.89-17.50]	16.88 [15.41-18.24]	14.51 [13.55-16.16]	<0.0001^a
	6	5.81 [5.03-6.68]	6.35 [5.76-7.23]	9.00 [7.97-10.01]	<0.0001^a
	7	6.90 [6.15-7.82]	7.45 [6.66-8.47]	14.59 [12.99-16.02]	<0.0001^a
	8	5.73 [4.93-6.70]	6.35 [5.47-7.32]	5.40 [4.65-6.00]	<0.0001^a
	9	6.79 [5.79-8.08]	7.45 [6.69-8.67]	6.53 [5.94-7.20]	<0.0001^a
	10	6.22 [5.41-7.36]	6.99 [6.16-7.92]	5.37 [4.59-6.03]	<0.0001^a
	11	5.76 [5.00-6.68]	6.42 [5.64-7.27]	6.30 [5.58-7.09]	<0.0001^a
	12	47.96 [43.89-51.86]	51.83 [48.75-54.61]	5.71 [5.01-6.32]	<0.0001^a
	13	22.40 [19.12-25.61]	25.61 [22.79-28.18]	5.18 [4.62-5.94]	<0.0001^a
	14	28.18 [24.27-32.45]	31.62 [28.82-35.46]	45.03 [41.22-47.49]	<0.0001^b
	15	10.08 [8.26-11.93]	11.85 [10.15-13.40]	19.88 [17.37-22.27]	<0.0001^a
<i>MAOA</i>	1	24.02 [16.44-44.62]	44.71 [41.08-48.85]	25.11 [22.01-27.14]	<0.0001^a
	2	15.03 [8.50-55.56]	55.67 [52.78-57.67]	8.47 [7.38-10.14]	<0.0001^a
	3	13.47 [7.68-32.34]	32.44 [27.80-34.41]	16.90 [14.38-19.93]	<0.0001^a
	4	9.84 [6.07-46.77]	46.99 [41.99-51.03]	8.67 [6.96-10.55]	<0.0001^b
	5	19.53 [10.47-54.88]	54.90 [52.32-57.55]	7.71 [6.06-9.48]	<0.0001^a
	6	13.30 [6.47-46.00]	46.09 [42.03-49.88]	6.19 [4.72-7.66]	<0.0001^a
	7	18.25 [12.41-60.73]	60.84 [58.69-63.01]	10.52 [8.98-12.45]	<0.0001^a
	8	17.50 [11.38-58.23]	58.36 [56.65-61.05]	6.55 [4.81-7.95]	<0.0001^a
	9	13.30 [9.20-21.74]	21.74 [19.06-24.57]	12.46 [10.26-14.49]	<0.0001^a
<i>HTR2A</i>	1	85.35 [81.91-87.07]	85.46 [79.39-89.98]	85.28 [82.13-87.26]	0.334 ^a
	2	89.67 [87.30-91.23]	90.02 [88.05-91.52]	88.90 [86.82-90.41]	0.004^a

37 Data are reported as median [interquartile range]. N, number of subjects. p-values for the difference between female and male
38 newborns were determined using ^aMann-Whitney test or ^bStudent's t-test. Statistically significant differences are in bold.
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Table S4. Bivariate associations between methylation of serotonin regulating genes in cord blood cells and other sample characteristics in PlaNS cohort.

	<i>SLC6A4</i> (average)	<i>MAOA</i> (average)	<i>HTR2A</i> (-1665)	<i>HTR2A</i> (-1224)
pBMI	0.161	0.720	0.979	0.263
GWG	0.038	0.385	0.022	0.213
GTS	0.869	0.737	0.407	0.543
PAR	0.017	0.628	0.329	0.420
MAD	0.053	0.146	0.269	0.947
TOB	0.482	0.618	0.053	0.623
SEX	<0.0001	<0.0001	0.334	0.004
GA	0.244	0.244	0.315	0.081
BW	0.043	0.008	0.558	0.019
PI	0.743	0.714	0.361	0.294

Shown are *p*-values for the association of *SLC6A4*, *MAOA*, and *HTR2A* methylation with neonatal sex (SEX), maternal pre-pregnancy body mass index (pBMI), gestational weight gain (GWG), glucose tolerance status (GTS; normoglycemia vs. gestational diabetes mellitus), parity (PAR; primiparous vs. multiparous), maternal age at childbirth (MAD), gestational age (GA), birth weight (BW), ponderal index (PI), and maternal smoking during pregnancy (TOB). Number of participants was 203, except for TOB, where it was 192 due to missing values. *p*-values were determined by Student's *t* test, Mann-Whitney test, Pearson correlation, or Spearman correlation, as appropriate. Statistically significant associations are in bold. In analyses separated by neonatal sex, no significant associations were found between *MAOA* methylation and any of maternal or neonatal characteristics.

Table S5. Bivariate associations between methylation of serotonin regulating genes in cord blood cells and cell type count in a subset of PlaNS participants (N=122).

	<i>SLC6A4</i> (average)	<i>MAOA</i> (average)	<i>HTR2A</i> (-1665)	<i>HTR2A</i> (-1224)
Lymphocytes	0.178	0.654	0.538	0.062
Monocytes	0.223	0.525	0.923	0.619
Neutrophils	0.166	0.043	0.706	0.416
Eosinophils	0.893	0.748	0.414	0.197
Basophils	0.365	0.707	0.371	0.150

Shown are *p*-values for the association of *SLC6A4*, *MAOA*, and *HTR2A* methylation with lymphocyte, monocyte, neutrophil, eosinophil and basophil counts in cord blood of subjects with available hematogram data (N=122). *p*-values were determined by Student's *t* test, Mann-Whitney test, Pearson correlation, or Spearman correlation, as appropriate. In analyses separated by neonatal sex, no significant associations were found between *MAO* methylation and any of cell type counts.

65 **Table S6.** Methylation levels (β values) in ARIES participants at CpG sites overlapping with CpG sites analyzed
66 in PlaNS cohort.

Gene	CpG site ^a	Time point	Integral sample	Females	Males	<i>p</i> -value
<i>SLC6A4</i>	#10	Birth	0.11 [0.08-0.13]	0.12 [0.09-0.14]	0.09 [0.08-0.12]	<0.0001 ^c
		7 years	0.15 [0.12-0.17]	0.15 [0.13-0.19]	0.15 [0.12-0.17]	<0.0001 ^c
		15 years	0.18 [0.16-0.21]	0.20 [0.17-0.22]	0.17 [0.15-0.20]	<0.0001 ^d
		<i>p</i> -value ^b	<0.0001	<0.0001	<0.0001	
	#14	Birth	0.33 [0.26-0.40]	0.37 [0.31-0.44]	0.28 [0.22-0.35]	<0.0001 ^c
		7 years	0.37 [0.31-0.44]	0.41 [0.36-0.48]	0.34 [0.28-0.40]	<0.0001 ^c
		15 years	0.43 [0.35-0.51]	0.48 [0.41-0.55]	0.38 [0.32-0.46]	<0.0001 ^c
		<i>p</i> -value ^b	<0.0001	<0.0001	<0.0001	
<i>HTR2A</i>	#1	Birth	0.73 [0.65-0.79]	0.73 [0.65-0.78]	0.74 [0.65-0.79]	0.375 ^c
		7 years	0.74 [0.66-0.78]	0.75 [0.67-0.78]	0.74 [0.67-0.77]	0.125 ^c
		15 years	0.70 [0.62-0.75]	0.70 [0.62-0.74]	0.70 [0.61-0.75]	0.722 ^c
		<i>p</i> -value ^b	<0.0001	<0.0001	<0.0001	
	#2	Birth	0.77 [0.75-0.80]	0.77 [0.75-0.80]	0.77 [0.75-0.80]	0.975 ^c
		7 years	0.72 [0.69-0.75]	0.72 [0.69-0.75]	0.73 [0.69-0.75]	0.109 ^c
		15 years	0.70 [0.65-0.73]	0.69 [0.65-0.72]	0.70 [0.65-0.73]	0.340 ^c
		<i>p</i> -value ^b	<0.0001	<0.0001	<0.0001	

67 Data are reported as median [interquartile range]. ^a CpG sites #10, #14, #1, and #2 correspond to Illumina IDs cg03363743,
68 cg22584138, cg02027079, and cg27068143, respectively. ^b*p*-values for the difference among different time points were
69 determined using Friedman test. *p*-values for the difference between female and male newborns were determined using ^cMann-
70 Whitney test or ^dStudent's *t*-test. Statistically significant differences are in bold.

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72 **Table S7.** Primers used in DNA methylation analyses by bisulfite pyrosequencing in PlaNS cohort.

Gene	CpG site #	Primer sequence (5' - 3')	GRCh38.p14/hg38 coordinates	Product size (bp)	Sequence source
<i>SLC6A4</i>	1–11	forward: GGGGAGGGGGATAGAAT	Chr17: 30 235 549 - 30 235 533	273	PyroMark Assay Design Software, version 2.0
		reverse (biotinylated): ACAACACTTTACTCAAAACCCTCTTTA	Chr17: 30 235 302 - 30 235 276		
		sequencing: GGGGAGGGGGATAGAAT	Chr17: 30 235 549 - 30 235 533		
	12–15	forward: TTTAAAGAGGGTTTTGAGTAAAGTG	Chr17: 30 235 306 - 30 235 281	150	PyroMark Assay Design Software, version 2.0
		reverse-biotinylated: TTTATATCAACCAAACTCTCCCTTTACAT	Chr17: 30 235 185 - 30 235 156		
		sequencing: GGGTTTTGAGTAAAGTGT	Chr17: 30 235 297 - 30 235 280		
<i>MAOA</i>	1–9	forward: GGGGAGTTGATAGAAGGGTTTTTTTAT	Chr23: 43 656 261 - 43 656 288	353	[1,2]
		reverse (biotinylated): TATATCTACCTCCCCAATCACACC	Chr23: 43 656 590 - 43 656 614		
		sequencing: AGTTAAAGTATGGAGAATTAAG	Chr23: 43 656 333 - 43 656 354		
<i>HTR2A</i>	1	forward: TGGTGGGGGAAAAAAATT	Chr13: 46 897 896 - 46 897 879	383	[3]
		reverse (biotinylated): AAATAACCTTTTATACAAATTCCC	Chr13: 46 897 236 - 46 897 213		
		sequencing: TGGTGGGGGAAAAAAATT	Chr13: 46 897 896 - 46 897 879		
	2	forward: GATATAAATATTGTTGGTTTTGGATGGA	Chr13: 46 897 377 - 46 897 850	276	[4]
		reverse (biotinylated): ACTACAAAATAACAACAACCAAAAA	Chr13: 46 897 125 - 46 897 101		
		sequencing: GGGAGAAGAAAAAGTTTG	Chr13: 46 897 155 - 46 897 138		

73 bp, base pair

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75 **References**

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