

## University of Groningen

### The first 1000 days and beyond

Küpers, Leanne Karen

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2016

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Küpers, L. K. (2016). *The first 1000 days and beyond: From early life environment to epigenetics and childhood overweight*. Rijksuniversiteit Groningen.

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*



# CHAPTER 4

# CORD BLOOD DNA METHYLATION AND BIRTH WEIGHT: A META-ANALYSIS OF EPIGENOME-WIDE ASSOCIATION STUDIES

Leanne K. Küpers\*, Janine F. Felix\*, Eva Corpeleijn and Harold Snieder,  
on behalf of the Pregnancy and Childhood Epigenetics (PACE) consortium.

*\* Equal contribution as first author*

## ABSTRACT

**Background:** We performed a meta-analysis of epigenome-wide association studies (meta-EWAS) in the Pregnancy and Child Epigenetics (PACE) consortium of multiple birth cohorts to identify differentially methylated Cytosine-phosphate-Guanine (CpG) sites in cord blood associated with birth weight.

**Methods:** This meta-EWAS was based on 6,027 newborns in 12 birth cohorts with DNA methylation in umbilical cord blood measured using the Infinium HumanMethylation450 BeadChip. All cohorts performed their EWAS based on a pre-specified analysis plan, with correction for covariates gestational age, gender, maternal social class, maternal smoking status, maternal age, maternal pre-pregnancy BMI, parity, batch and estimated cell type proportions. We performed a fixed effects inverse variance meta-analysis and used a Bonferroni corrected threshold of  $P < 1.055 \times 10^{-7}$ . We analyzed two models: one with birth weight on a continuous scale and one with birth weight as a dichotomous trait (high versus normal birth weight).

**Results:** We found 490 CpGs in 293 genes associated with the continuous measure of birth weight. 279 of the total of 293 genes (95%) had not previously been reported to be associated with birth weight. The *WNT1*, *LOXL1* and *PRDM16* genes had the highest number of significant CpGs with 8, 7 and 5 CpGs mapping to these genes, respectively. The ten genes with the most significant CpGs were *UHRF1*, *ARID5B*, *KLF9*, *TSC2*, *XRCC3*, *PDE4D*, *PIM1*, *KCNC4*, *YPEL3* and *DAB2IP*. We found 58 CpGs in the dichotomous birth weight model, of which 49 (84.5%) CpGs were also found in the continuous model.

**Conclusion:** This first meta-analysis of multiple EWASs for birth weight yielded a steep increase in the number of genes showing differential methylation associated with birth weight. This large list of differentially methylated genes can be used to improve the insight in the biological mechanisms underlying the relation between intrauterine exposures and adult health.

## INTRODUCTION

Birth weight is an important measure of intrauterine growth and development and it can be considered to reflect the effect of all (adverse) intrauterine exposures combined, such as maternal smoking during pregnancy, gestational diabetes and stress during pregnancy<sup>1-3</sup>. In the developmental origins of health and disease (DOHaD) hypothesis, a low and a high birth weight have been associated with cardiometabolic complications during childhood and adulthood<sup>4,5</sup>. Low birth weight could also be a predictor of accelerated childhood growth<sup>6</sup> and overweight later in life<sup>7</sup>.

An important biological mechanism that is hypothesized to play a role in the association between adverse exposures and intrauterine development is DNA methylation. This was confirmed by several epigenome-wide association studies (EWAS) that showed an association between specific intrauterine exposures (e.g. smoking during pregnancy) and differential DNA methylation in cord blood of the offspring<sup>8-13</sup>. Differential DNA methylation has also been studied for its association with birth weight in both candidate-gene<sup>14-17</sup> and epigenome-wide association studies<sup>9,18,18-20</sup>, however no clear consensus was found in their results. In the epigenome-wide studies for example, Adkins et al. analyzed the cord blood of 201 newborns using the Illumina 27k chip, and they were not able to identify any significant epigenome-wide associations of DNA methylation with birth weight<sup>18</sup>. On the contrary, Engel et al.<sup>9</sup> found 19 Cytosine-phosphate-Guanine (CpG) sites to be associated with birth weight using Illumina's 450k chip in cord blood of 1,046 Norwegian newborns. However, these results were not replicated in another cohort. In addition, using the 450k chip, we have recently identified some CpGs in the *GFI1* gene to be mediating the association between maternal smoking during pregnancy and birth weight of the offspring<sup>11</sup>. This mediating effect was found in cord blood of 255 newborns and replicated in a group of 1423 newborns. However, this study investigated a mediation model to follow up results of maternal smoking and was not an EWAS specifically focused on finding novel CpGs associated with birth weight. Other studies analyzed DNA methylation levels in adult blood samples, which also did not show consistent evidence for associations with birth weight<sup>20,21</sup>. Therefore, we decided to conduct a large meta-analysis of epigenome-wide association studies (meta-EWAS) aiming to clarify the association between cord blood DNA methylation and birth weight.

Combining multiple epigenome-wide association studies into a meta-analysis is important, because it increases the power to identify novel genomic regions showing

differential methylation significantly associated with birth weight. The success of these meta-analyses has extensively been shown in genetic epidemiology. This approach also enabled us to perform subgroup analyses that are often not possible in individual studies because of the small numbers of cases. Here, we performed a meta-EWAS on continuous birth weight, as well as a comparison between birth weight categories in the Pregnancy and Child Epigenetics (PACE) consortium of multiple birth cohorts aiming to identify differentially methylated CpG sites (or genes) in cord blood (measured using the Infinium HumanMethylation450 BeadChip) associated with birth weight.

## METHODS

### *Cohorts and subjects*

This meta-EWAS was based on 12 birth cohorts in the US and Europe with DNA methylation measured in umbilical cord blood. These studies, listed in alphabetic order are the Avon Longitudinal Study of Parents and Children (ALSPAC), the Children's Health Study (CHS), the GECKO Drenthe cohort, the Generation R Study, the Genetics of Overweight Young Adults (GOYA) study, Infancia y Medio Ambiente (INMA), three independent datasets from the Norwegian Mother and Child Cohort Study (MoBa1, MoBa2, and MoBa3), the Norway Facial Clefts Study (NCL), the Newborn Epigenetics Study (NEST) and the PRISM cohort. A total of 6,027 newborns were included in this meta-analysis. Details of the participating cohorts are summarized in **Supplementary Table 1**. Birth weight was analyzed as a continuous measure expressed in grams, as well as dichotomously comparing high birth weight (>4000g) with normal birth weight (2500-4000g). A subgroup analysis in low birth weight newborns was not possible because we excluded preterm newborns (<37 weeks), which is the largest group of low birth weight newborns. This resulted in too few low birth weight newborns for the meta-EWAS. For most cohorts, birth weight was objectively measured and extracted from medical files. Data collection and DNA analysis was approved by medical ethics committees for all participating cohorts and all participating parents gave (written) informed consent.

### *Epigenome-wide association studies*

Epigenome-wide association with birth weight was analyzed in a total of 6,027 newborns. All cohorts performed bisulfite conversion using the EZ-96 DNA methylation kit (Zymo research Corporation, Irvine, USA). Subsequently, all cohorts used the Illumina HumanMethylation450 (450k) Beadchip (Illumina Inc., San Diego, USA) to

measure methylation levels and in the meta-analysis we analysed a total of 473,855 CpGs. All cohorts performed their EWAS based on a pre-specified analysis plan. This plan included a definition of covariates: gestational age, gender, maternal social class, maternal smoking status, maternal age, maternal pre-pregnancy BMI, parity, batch and estimated cell proportions. Cell proportions were estimated using the method previously described by Houseman and colleagues<sup>22</sup> with the dataset of Reinius and colleagues<sup>23</sup>. We used the cell types (B cells, granulocytes, monocytes, NK cells, CD4+ cells and CD8+ cells) as covariates in the model. Optionally, the NEST and PRISM cohorts also adjusted for ancestry and NCL adjusted for cohort-specific selection factors. We performed complete case analyses, meaning that cases with missing data on birth weight or one of the covariates were excluded from analyses. Additionally, to remove extreme methylation outliers that could adversely influence the results, all cohorts trimmed the methylation data of all CpGs on:

(25th percentile -3\*IQR) and (75th percentile+3\*IQR)

In which IQR = interquartile range. This means that for each probe values outside this range were set to NA in the methylation set. Robust linear regression modelling was performed for continuous birth weight as outcome, and an adjusted generalized linear model was performed for the binary outcome of high birth weight versus normal birth weight in *R*. We tested the following models both before and after correction for estimated cell proportions:

- 1 Birth weight (continuous) = Methylation + gestational age + gender + maternal social class + maternal smoking + maternal age + maternal pre-pregnancy BMI + parity + batch (+ ancestry)(+ selection factors) (+ estimated cell proportions)
- 2 Birth weight (binary, high BW versus normal BW) = Methylation + gestational age + gender + maternal social class + maternal smoking + maternal age + maternal pre-pregnancy BMI + parity + batch (+ ancestry)(+ selection factors) (+ estimated cell proportions)

### **Quality control**

Quality control of samples was first performed by each individual cohort and included the exclusion of failed samples based on Illumina's detection P-value, low sample DNA concentration, sample call rate, CpG specific percentage of missing values, bisulfite conversion efficiency, gender verification with multidimensional scaling plots, and other quality control metrics specific to cohorts. Subsequently, before performing the meta-analysis, we performed quality control to check compatibility of the results among all cohorts. During this quality control for each individual cohort we performed checks of

the distribution of effect sizes and standard errors, a correlation plot of actual p-values against p-values calculated from the beta and standard error, a QQ plot for over/undersignificance, a Manhattan plot for location of signals and a volcano plot for the distribution of effect sizes and corresponding p-values. Additionally, we constructed an overall precision plot to compare the distribution of precision ( $1 / \text{median}(\text{SE})$ ) against the square root of the sample size for each cohort. Finally, we plotted distributions of effect sizes for all cohorts for a comparison of unit of effect, this allowed us to detect whether some cohorts inadvertently used different units of measurement. In that case, the cohort was asked to redo the analysis. We conducted all these quality control analyses in our own R package "QCEWAS" that we specifically developed for quality control of meta-EWAS.

### ***Meta-analysis***

We performed a fixed effects inverse variance meta-analysis, using METAL<sup>24</sup>. To correct for multiple testing we set the Bonferroni genome-wide significance threshold at  $P < 1.055 \times 10^{-7}$  ( $0.05 / 473,855$  CpGs). Additionally, we calculated the  $I^2$  with corresponding p-value as a measure of heterogeneity between cohorts.

## **RESULTS**

In total we included 6,027 newborns from 12 cohorts in this meta-analysis on the association between cord blood DNA methylation and birth weight. Cohort-specific characteristics are presented in **Table 1** and more details can be found in **Supplementary Table 1**. The mean birth weight of these infants in the different cohorts ranged from 3297 to 3750 grams. In this total meta-EWAS 4,392 newborns (78%) had a normal birth weight between 2,500 and 4,000 grams and 1,246 (22%) newborns were born with a high birth weight of more than 4,000 grams. We excluded preterm newborns, which resulted in too few newborns (only MoBa1 and NEST had more than 15 low birth weight newborns per cohort) with a low birth weight for this meta-analysis.

In cohort-specific quality control we observed that all cohorts were comparable and compatible for meta-EWAS. All cohorts showed proper QQ plots, plausible Volcano plots and Manhattan plots and comparable distributions of effect sizes and standard errors.



We analyzed two models: a model with birth weight on a continuous scale and a model with birth weight as a dichotomous trait (high versus normal birth weight). The results of the continuous model are presented in **Table 2**, in the Manhattan plot in **Figure 1** and the Volcano plot in **Figure 2**. In this meta-analysis of 12 EWASs we observed that 490 CpGs were significantly associated with the continuous measure of birth weight after Bonferroni correction for multiple testing. Of the 490 Bonferroni significant CpGs, 188 (38.4%) were negatively associated with birth weight, for these CpGs higher methylation levels went with a lower birth weight. The remaining 302 CpGs (61.6%) had a positive association with birth weight, i.e., higher methylation levels went with a higher birth weight for these CpGs (**Figure 2**). Overall, this meta-EWAS with continuous birth weight as the outcome resulted in a maximum negative CpG-specific effect size of -73.04g difference in birth weight for 1% change in methylation and a maximum positive effect size of 93.58g difference in birth weight for 1% change in methylation. The overall lambda for this meta-analysis was 1.58. The 490 top CpGs mapped to 293 genes. The *WNT1*, *LOXL1* and *PRDM16* genes had the largest number of significant CpGs with 8, 7 and 5 CpGs mapping to these genes, respectively. The top CpG site in our meta-EWAS, cg17714703 located in the *UHRF1* gene, had a significance level of  $P = 4.7 \cdot 10^{-32}$ . The ten genes with the lowest p-values were *UHRF1*, *ARID5B*, *KLF9*, *TSC2*, *XRCC3*, *PDE4D*, *PIM1*, *KCNC4*, *YPEL3* and *DAB2IP*. A total of 117 CpGs were not annotated within the boundaries of a gene, using the UCSC annotation data.

The results from the dichotomous model, comparing high birth weight with normal birth weight, are presented in **Supplementary Table 2**. This meta-EWAS resulted in 58 CpGs with significant p-value after Bonferroni correction for multiple testing. These 58 CpGs mapped to 36 genes. Only the *ARID5B* had two significant CpGs mapping to the gene, which were both in the top 6 CpGs with the lowest p-values. The rest of the genes had only one Bonferroni significant CpG per gene. A total of 20 CpGs were not annotated within the boundaries of a gene, using the UCSC annotation data. Of these 58 CpGs, 44 (75.9%) CpGs were negatively associated with birth weight and 14 (24.1%) CpGs were positively associated with birth weight. Of all 58 CpGs in this dichotomous model, 49 (84.5%) were also found in the 490 Bonferroni significant CpGs of the continuous model.

**TABLE 1.** Birth weight distribution and frequencies for all cohorts.

<b>Study</b>	<b>N†</b>	<b>Birth weight in grams Mean (SD)</b>	<b>Normal birth weight ‡ N (%)</b>	<b>High birth weight ‡ N (%)</b>	<b>Low birth weight ‡ N (%)</b>
ALSPAC	633	3511.80 (442.7)	547 (86.4%)	79 (12.5%)	7 (1.1%)
CHS	199	3486.00 (476.0)	168 (84.4%)	28 (14.1%)	3 (1.5%)
GECKO	255	3542.77 (533.0)	206 (80.8%)	46 (18.0%)	3 (1.2%)
GENERATION R	717	3571.68 (464.48)	589 (82%)	122 (17%)	6 (1%)
GOYA	947	3750.00 (501.5)	649 (68.5%)	294 (31.1%)	4 (0.4%)
INMA*	166	3296.75 (400.2)	156 (94.0%)	7 (4.2%)	3 (1.8%)
MOBA1	1025	3644.34 (543.6)	754 (73.6%)	243 (23.7%)	28 (2.7%)
MOBA2	587	3700.80 (487.0)	435 (74.1%)	146 (24.9%)	6 (1.0%)
MOBA3	205	3705.52 (490.5)	153 (74.6%)	51 (24.9%)	1 (0.5%)
NCL	802	3670.00 (505.0)	601 (74.9%)	193 (24.1%)	8 (1.0%)
NEST	267	3323.00 (517.0)	226 (84.6%)	24 (9.0%)	17 (6.4%)
PRISM*	138	3384.64 (440.6)	122 (88.4%)	12 (8.7%)	4 (2.9%)

† N=number of participants with birth weight data, 450K methylation, and covariates. Participants with a low birth weight were excluded from the analyses with dichotomous outcome high birth weight versus normal birth weight.

‡ Normal birth weight: 2500-4000 grams and high birth weight: >4000 grams and low birth weight: <2500 grams.

\* Cohorts where the high birth weight category had N<15, resulting in exclusion from these analyses.

**TABLE 2.** Meta-analysis results with Bonferroni corrected genome-wide significant differential DNA methylation in relation to birth weight on a continuous scale, sorted on p value.

CpG	Effect size	SE	P	Direction	Mapped gene	CHR	Location	BP position	Location
cg17714703	1588.92	134.84	4.71E-32	+++++-----	UHRF1	19	Body	4912221	Shore
cg25953130	-786.15	75.26	1.52E-25	-----+---	ARID5B	10	Body	63753550	Opensea
cg20076442	-908.61	90.66	1.22E-23	-----		8		72745197	Opensea
cg00049440	-975.55	97.50	1.44E-23	-----+---	KLF9	9	Body	73026643	Shore
cg08404702	998.19	99.78	1.47E-23	+++++-----	TSC2	16	Body	2133388	Shore
cg04872675	1182.45	120.34	8.69E-23	+++++-----		12		108983234	Opensea
cg25124943	-1106.15	115.35	8.86E-22	---?-----		10		4117248	Opensea
cg12798040	1134.34	121.92	1.36E-20	+++++-----	XRCC3	14	Body	104171840	Opensea
cg07144560	-1289.93	139.85	2.87E-20	---?--+-----		5		148865756	Opensea
cg18804667	648.66	73.31	8.90E-19	+++++-----	PDE4D	5	Body	58883392	Opensea
cg25325512	634.71	72.64	2.37E-18	+++++-----	PIM1	6	3'UTR	37142220	Shelf
cg12723026	709.60	82.62	8.78E-18	+++++-----	KCNC4	1	Body	110764594	Shore
cg02863179	-638.83	74.41	9.04E-18	-----+---	ARID5B	10	Body	63779053	Opensea
cg27106909	1598.53	187.38	1.45E-17	+++++-----	YPEL3	16	1stExon	30106897	Shore
cg13758780	1201.76	141.22	1.74E-17	+++++-----	DAB2IP	9	Body	124329514	Opensea
cg02149899	1203.00	144.19	7.23E-17	+++++-----		11		75993521	Opensea
cg00472758	1238.31	149.40	1.15E-16	+++++-----	TBC1D24	16	3'UTR	2552820	Shelf
cg11138362	1159.49	139.91	1.16E-16	+++?+++++?	PRDM16	1	Body	3239992	Island
cg25162533	831.26	101.23	2.18E-16	+++++-----		17		81032067	Shore
cg15283950	1053.65	132.65	1.98E-15	+++++-----	MRV1	11	1stExon	10715217	Opensea
cg14651616	1757.10	222.03	2.49E-15	+++++-----	MAP4K2	11	Body	64563992	Opensea
cg22877366	781.59	98.90	2.73E-15	-+++++-----		1		12107668	Opensea
cg17836177	1359.10	173.11	4.12E-15	+++++-----	PEBP4	8	Body	22735111	Opensea
cg24559796	-905.25	115.60	4.84E-15	-----+---		7		28223298	Shelf
cg15233611	1159.44	148.15	5.03E-15	+++++-----	SETD1B	12	Body	122244660	Shore
cg05993265	1380.38	176.55	5.34E-15	+++++-----	MFS10	4	Body	2933802	Island
cg13280041	969.34	124.02	5.44E-15	+++++-----	NHSL1	6	Body	138866865	Opensea
cg20652404	1082.63	139.11	7.11E-15	+++++-----	LOXL1	15	5'UTR	74218904	Island
cg23414595	820.07	105.65	8.38E-15	+++++-----	ZMAT5	22	Body	30129607	Shelf
cg19379721	1046.91	135.26	9.93E-15	+++?+++++	LPAR2	19	Body	19737143	Shore
cg09695430	-921.86	119.16	1.03E-14	-----	WT1	11	Body	32447944	Shore
cg24324628	1187.80	153.69	1.09E-14	+++++-----	NHSL1	6	Body	138866882	Opensea
cg00055726	1312.76	169.91	1.11E-14	?++++?++?++	FAM163B	9	TSS200	136445425	Shore
cg11268834	1074.65	139.16	1.14E-14	+++++-----	MRPL44	2	Body	224824500	Shore
cg22590761	1136.91	147.58	1.32E-14	+++++-----	LOXL1	15	5'UTR	74218921	Island
cg17850088	709.37	92.19	1.42E-14	+++++-----?		1		150119278	Shelf
cg14292823	1569.59	204.52	1.66E-14	+++++-----	EEF1D	8	TSS1500	144681097	Island
cg12022621	-880.95	115.15	2.01E-14	-----+---	LAX1	1	1stExon	203734505	Opensea
cg01418153	928.57	121.66	2.30E-14	+++?+++++	PRDM16	1	Body	3240129	Island
cg27217742	1113.55	146.45	2.89E-14	+++++-----	RGS12	4	Body	3365280	Island
cg13904214	1027.96	135.74	3.65E-14	+++++-----	RBM33	7	Body	155556729	Opensea
cg23902550	1776.03	234.52	3.65E-14	+++++-----	EEF1D	8	TSS1500	144681106	Island
cg12781678	949.46	125.40	3.70E-14	+++++-----		7		100053754	Opensea
cg00376553	921.87	122.23	4.63E-14	+++++-----	TSC22D4	7	Body	100073281	Shelf
cg11303839	-481.67	63.90	4.80E-14	-----	CCL26	7	5'UTR	75405967	Opensea
cg05779272	1918.94	254.83	5.06E-14	+++++-----	B4GALT1	9	TSS1500	33168018	Shore
cg00531137	-829.69	110.26	5.28E-14	-----+---		16		57643932	Opensea
cg10372921	937.84	124.72	5.49E-14	+++++-----	LOXL1	15	TSS200	74218733	Island
cg00028013	940.64	125.35	6.17E-14	+++++-----	LOXL1	15	TSS200	74218697	Island
cg04968127	1073.35	143.07	6.27E-14	+++++-----	MRV1	11	1stExon	10715213	Opensea
cg02232751	1660.92	221.60	6.62E-14	+++?+++++	LDHA	11	TSS200	18415742	Shore
cg01715745	-1006.37	134.78	8.22E-14	---?-----		1		54930156	Opensea
cg14097568	-755.78	101.46	9.42E-14	-----+---		1		94792942	Opensea
cg05575921	981.18	131.89	1.01E-13	+++++-----	AHRR	5	Body	373378	Shore
cg07313882	1503.72	202.47	1.11E-13	+++++-----	TMC6	17	Body	76121348	Opensea
cg13492133	805.27	108.58	1.21E-13	+++++-----	SMG7	1	Body	183516266	Opensea
cg09321238	1629.42	220.13	1.34E-13	+++?+++++	PRDM16	1	Body	3072001	Island
cg05304729	724.13	98.07	1.54E-13	+++++-----	MNDA	1	TSS1500	158800024	Opensea
cg10829227	1021.92	138.46	1.58E-13	+++++-----	PRKD2	19	Body	47200595	Shelf
cg26191447	-943.68	128.35	1.95E-13	-----+---		2		191656418	Opensea
cg07677157	-581.91	79.23	2.07E-13	---?-----?		12		66050928	Opensea
cg24693803	862.71	117.82	2.43E-13	+++?++?++++		4		171013537	Shelf
cg13719443	1249.17	170.69	2.51E-13	+++++-----	LOC285847	6	Body	35700382	Shore
cg16348385	1620.27	221.52	2.59E-13	+++++-----	YPEL3	16	1stExon	30106822	Shore

4

**TABLE 2.** Meta-analysis results with Bonferroni corrected genome-wide significant differential DNA methylation in relation to birth weight on a continuous scale, sorted on p value. *Continued*

CpG	Effect size	SE	P	Direction	Mapped gene	CHR	Location	BP position	Location
cg25164589	-807.50	110.40	2.59E-13	-----+--		10		63540024	Opensea
cg20752878	874.54	119.81	2.89E-13	+++++	ASGR1	17	Body	7080538	Shelf
cg13660174	908.22	125.48	4.55E-13	+++++	SURF4	9	Body	136238392	Shelf
cg21516291	1242.95	172.67	6.09E-13	+++++	SLC35C2	20	Body	44979100	Opensea
cg21995347	760.44	105.73	6.38E-13	+++++	SNX18	5	1stExon	53814891	Island
cg07397493	1043.38	145.10	6.45E-13	+++++	LOC100133545	11	TSS200	2011312	Shelf
cg15542713	468.06	65.20	7.01E-13	++-?+-----	HIVEP3	1	TSS1500	42385581	Shore
cg15681239	-601.81	84.12	8.41E-13	--?-+-----	DLEC1	3	TSS1500	38080203	Shore
cg10117369	-847.84	118.75	9.34E-13	-----+---	LAX1	1	TSS200	203734256	Opensea
cg12573002	-1517.38	213.10	1.07E-12	-+-----+---	NCR3	6	5'UTR	31560624	Opensea
cg02983090	-705.51	99.17	1.13E-12	-----+---	IL21R	16	TSS1500	27437892	Opensea
cg20219891	-260.15	36.57	1.13E-12	-+-----+---		2		121496875	Shore
cg00587472	-671.74	94.71	1.32E-12	-----+---		2		6952860	Opensea
cg26438325	-923.70	130.39	1.40E-12	--?-+-----	JAZF1	7	Body	28176718	Opensea
cg06673684	624.53	88.33	1.54E-12	+++++	MCF2L	13	Body	113646815	Shore
cg13586051	-857.88	121.55	1.69E-12	-----+---	DNASE1L3	3	TSS200	58200471	Opensea
cg13641903	-811.14	115.17	1.88E-12	-----+---	WT1	11	Body	32452608	Island
cg06378491	1945.17	277.11	2.23E-12	+++++	MAP4K2	11	Body	64564012	Opensea
cg23917006	995.11	141.77	2.23E-12	+++++	CLDN19	1	TSS1500	43207294	Shelf
cg00329157	-1193.65	170.31	2.41E-12	-----+---	GCET2	3	5'UTR	111844870	Opensea
cg17782974	707.30	101.36	3.00E-12	+++++	TRIM8	10	Body	104406990	Shelf
cg12804755	-572.79	82.14	3.09E-12	-----+---		11		15796715	Opensea
cg08005122	-1498.63	215.15	3.28E-12	-----+---		11		110779637	Opensea
cg10317026	-1906.85	275.56	4.52E-12	-----+---	EFNA3	1	Body	155056636	Shore
cg21058822	-590.10	85.40	4.87E-12	-?------	MBOAT4	8	TSS200	30002223	Opensea
cg12259522	1217.56	176.82	5.74E-12	+++++		13		112310720	Opensea
cg16145216	701.10	102.03	6.36E-12	+++++	HIVEP3	1	TSS1500	42385662	Shore
cg23237276	657.72	96.08	7.63E-12	+++++		14		101827449	Shelf
cg03486991	-795.12	116.33	8.21E-12	--?-+-----		2		127975738	Shore
cg13872898	-295.22	43.25	8.71E-12	-----+---		2		121498194	Shore
cg00395063	1241.21	181.97	9.04E-12	-----+---	ARHGEF12	11	Body	120210293	Shelf
cg16014606	921.16	135.25	9.71E-12	+++++	MRV11	11	1stExon	10715221	Opensea
cg19260606	-589.38	86.57	9.89E-12	--?-+-----	KIAA1026	1	Body	15100030	Opensea
cg12594244	1220.58	179.50	1.05E-11	+++++	LOXL1	15	TSS200	74218754	Island
cg11701868	844.13	124.25	1.09E-11	++?+++-----	UHRF1	19	Body	4912006	Shore
cg18536994	891.50	131.23	1.10E-11	+++++	LOC283999	17	Body	76230868	Shelf
cg04070976	960.58	141.46	1.12E-11	-+?+++-----		17		73577422	Opensea
cg11334730	1098.73	162.22	1.26E-11	+++++		6		6750455	Opensea
cg23719157	512.16	75.71	1.34E-11	+++++	STRA6	15	TSS1500	74501584	Opensea
cg15806306	-1092.22	162.23	1.67E-11	-----+---		11		2893385	Shelf
cg03173827	1209.13	179.83	1.77E-11	+++++	TCEA2	20	1stExon	62688717	Island
cg14035521	1474.40	219.60	1.89E-11	+++++	POLR1E	9	Body	37486469	Shore
cg14096899	589.14	87.92	2.07E-11	+++++		2		240697719	Opensea
cg14774305	-1430.32	213.74	2.20E-11	-----+---		5		137430567	Opensea
cg05387269	841.22	125.75	2.24E-11	++-+-----		1		1306946	Shelf
cg17353337	849.69	127.03	2.25E-11	+++++	NHSL1	6	Body	138866898	Opensea
cg22246785	-855.08	127.85	2.26E-11	-----+---		12		122021013	Shelf
cg03996020	557.72	83.47	2.36E-11	+++++	DNAJB6	7	Body	157180124	Opensea
cg17298279	1835.64	276.10	2.96E-11	-+-----	TMEM181	6	Body	158982078	Shore
cg05851442	643.71	96.83	2.97E-11	+++++	HXA3	7	5'UTR	27153212	Island
cg03527802	1017.70	153.13	3.01E-11	+++++	MAZ	16	Body	29819931	Shore
cg12036877	782.47	117.81	3.10E-11	+++++	BGLAP	1	TSS200	156211896	Shelf
cg01919885	817.08	123.04	3.12E-11	+++++	RGS12	4	Body	3365330	Island
cg08323969	1072.96	161.64	3.18E-11	+++++		17		40770823	Opensea
cg14435807	1148.63	173.11	3.24E-11	+++++	LOXL1	15	TSS200	74218780	Island
cg23032421	-507.96	76.60	3.33E-11	-----+---	IL5RA	3	5'UTR	3152038	Opensea
cg24019564	1045.76	157.70	3.33E-11	+++++	RUNX3	1	TSS1500	25257566	Island
cg08282819	-723.82	109.24	3.45E-11	-----+---	IL21R	16	TSS1500	27437900	Opensea
cg17758673	656.81	99.49	4.06E-11	+++++	RGS12	4	Body	3365342	Island
cg10453071	997.35	151.08	4.07E-11	+++++	DOK4	16	Body	57513414	Opensea
cg14548802	673.09	101.98	4.11E-11	+++++	COL5A1	9	Body	137675380	Opensea
cg15700009	1012.55	153.90	4.72E-11	+++++	LDHA	11	TSS1500	18415711	Shore
cg00637745	-246.09	37.42	4.80E-11	-+-----		2		121497334	Shore

**TABLE 2.** Meta-analysis results with Bonferroni corrected genome-wide significant differential DNA methylation in relation to birth weight on a continuous scale, sorted on p value. *Continued*

CpG	Effect size	SE	P	Direction	Mapped gene	CHR	Location	BP position	Location
cg11370064	639.43	97.22	4.80E-11	+++?++++++		3		78318277	Opensea
cg27636559	-1171.73	178.32	5.01E-11	-----+--	EFTUD1	15	Body	82431147	Opensea
cg26432350	1141.69	173.78	5.03E-11	+++++++	NBEAL2	3	Body	47040357	Opensea
cg09294084	659.46	100.50	5.33E-11	++++++	MCF2L	13	Body	113646732	Shore
cg19211915	-1063.15	162.04	5.35E-11	+--+-----	WT1	11	Body	32452513	Island
cg26828842	-783.99	119.54	5.44E-11	--+-----		18		67952086	Shelf
cg15995125	972.55	149.15	7.00E-11	+++++++	RCC2	1	Body	17746597	Shore
cg21221767	-883.81	135.56	7.05E-11	--+-----		14		65673646	Opensea
cg23494863	-944.35	144.88	7.13E-11	-----+--	STAR	8	TSS1500	38009476	Opensea
cg04479212	761.08	116.80	7.23E-11	+++++++	ITGA9	3	Body	37495166	Shore
cg17870997	-424.30	65.25	7.90E-11	+-----+		2		121498521	Shore
cg10073091	-1890.65	291.06	8.26E-11	-----+--	DHCR24	1	Body	55352301	Shore
cg03162410	1360.55	209.46	8.28E-11	+++++++	TECR	19	Body	14671422	Opensea
cg05950943	-1525.72	235.02	8.48E-11	+-----	NTN3	16	TSS1500	2520971	Shore
cg19242268	1095.40	168.80	8.61E-11	+++++++	TCEA2	20	1stExon	62688573	Island
cg00218409	-1482.66	228.58	8.79E-11	-----		2		74963008	Opensea
cg17711527	-797.31	122.96	8.91E-11	-----+--	LAX1	1	1stExon	203734396	Opensea
cg13120986	760.51	117.43	9.41E-11	+++++++	C15	12	1stExon	7168012	Opensea
cg20782117	-736.48	113.75	9.52E-11	-----+--	SLC25A13	7	Body	95865631	Opensea
cg20068209	-576.01	89.00	9.68E-11	--?-+--+	TMEM30A	6	Body	75988568	Opensea
cg05056497	-503.37	77.91	1.04E-10	--+-----	RCAN1	21	5'UTR	35899448	Opensea
cg12149606	635.87	98.51	1.08E-10	+++++++	TMED10	14	Body	75614186	Opensea
cg02194129	656.03	101.80	1.16E-10	+++++++	XRCC3	14	Body	104171313	Opensea
cg20449670	-754.97	117.43	1.29E-10	--?-+--+		1		153498959	Opensea
cg26950531	-579.94	90.24	1.30E-10	-----+--	DPF1	19	Body	38704515	Shelf
cg13078421	721.50	112.44	1.39E-10	++++++	GNCG	19	5'UTR	2624622	Shore
cg13696490	-615.98	96.00	1.39E-10	-----+--	LOC201651	3	TSS1500	151488006	Opensea
cg00942920	-699.10	109.04	1.44E-10	-----	LAX1	1	Body	203734559	Opensea
cg03254465	609.84	95.12	1.45E-10	+++?+++++	PRDM16	1	Body	3240227	Island
cg20664313	841.45	131.32	1.48E-10	+++++++	FBRSL1	12	Body	133071297	Shelf
cg01063280	-850.28	132.76	1.51E-10	--?-+--+		6		108167599	Shore
cg23663547	1130.95	176.62	1.52E-10	+++++++	CCR7	17	3'UTR	38710320	Opensea
cg21611682	895.58	139.89	1.53E-10	?+++++	LRP5	11	Body	68138269	Opensea
cg04310331	1504.68	236.17	1.88E-10	+++++++	LOC100133545	11	TSS1500	2012332	Shore
cg18428006	1211.34	190.28	1.94E-10	+++?+++++	HNRNPAB	5	Body	177633449	Shore
cg13687834	-592.87	93.31	2.10E-10	-----		10		3514783	Opensea
cg08080174	-1030.36	162.29	2.17E-10	--+-----	ZFXH3	16	Body	72992580	Shore
cg13742400	-938.11	147.90	2.26E-10	+-----+	DOCK10	2	Body	225639708	Opensea
cg18299068	854.77	134.83	2.30E-10	+++++++	MAEA	4	Body	1305425	Shore
cg20948740	1175.09	185.50	2.38E-10	+++++++	DNMT3A	2	Body	25475726	Island
cg15962969	854.35	134.91	2.41E-10	+++++++	NHSL1	6	Body	138866887	Opensea
cg14195992	1016.14	160.57	2.48E-10	+++++++	KIAA0146	8	Body	48265917	Opensea
cg02402882	-847.57	134.07	2.58E-10	-----		5		56620429	Opensea
cg22030032	1315.20	208.50	2.83E-10	+++++++	EGLN2	19	Body	41312792	Shelf
cg20359445	979.03	155.25	2.86E-10	+++++++	IKZF4	12	Body	56415591	Opensea
cg07168232	-733.96	116.49	2.96E-10	-----	LAMB3	1	Body	209822928	Opensea
cg16014412	1439.38	228.57	3.03E-10	++?+++++		1		9687471	Shore
cg14311362	-499.74	79.38	3.06E-10	+-----+		2		121498812	Shore
cg04228709	590.83	93.85	3.07E-10	++++++	TIAM2	6	Body	155537930	Shelf
cg11950754	810.03	129.01	3.41E-10	+++++++	LRP8	1	Body	53782077	Opensea
cg11171811	1029.00	164.18	3.66E-10	+++++++	LTBP3	11	TSS1500	65326725	Shore
cg17520654	-797.49	127.51	3.99E-10	-----		12		13409474	Opensea
cg27115863	-1127.23	180.53	4.27E-10	-----+--		22		37921640	Opensea
cg14459032	-783.47	125.51	4.31E-10	--?-+--+		9		78905335	Opensea
cg01515508	792.32	127.01	4.43E-10	+++++++	CASZ1	1	Body	10728270	Shelf
cg14875081	1349.00	216.67	4.78E-10	+++++++		2		96814660	Shelf
cg21805179	580.40	93.25	4.85E-10	+++?+++++	MPHOSPH9	12	TSS1500	123707825	Opensea
cg05925497	-619.98	99.62	4.7E-10	-----	FLJ32810	11	Body	100734094	Opensea
cg27246744	-932.68	150.23	5.36E-10	-----+--	OSBPL10	3	Body	31746590	Opensea
cg24049493	598.18	96.48	5.64E-10	+++++?	HIVEP3	1	TSS1500	42385941	Shore
cg19448816	-786.43	126.96	5.86E-10	-----+--	PLCL2	3	Body	16974217	Opensea
cg01906801	826.87	133.64	6.12E-10	+++++++	MED24	17	Body	38191472	Opensea
cg07317017	979.71	158.39	6.20E-10	+++++++	LOXL1	15	TSS200	74218715	Island

4

**TABLE 2.** Meta-analysis results with Bonferroni corrected genome-wide significant differential DNA methylation in relation to birth weight on a continuous scale, sorted on p value. *Continued*

CpG	Effect size	SE	P	Direction	Mapped gene	CHR	Location	BP position	Location
cg18739367	-1052.02	170.14	6.27E-10	+--+-----		8		38330740	Opensea
cg07340025	-574.31	92.88	6.28E-10	-----++		5		14926200	Opensea
cg09726279	1331.72	215.43	6.35E-10	+++++	MYBBP1A	17	Body	4457835	Shore
cg00442282	-1431.87	231.84	6.57E-10	-----+--	RARA	17	5'UTR	38471064	Shore
cg16476991	1078.25	174.63	6.64E-10	+++++	RASA3	13	Body	114831404	Opensea
cg15165154	648.45	105.11	6.85E-10	+++++	CXCC5	5	5'UTR	139041014	Island
cg00153395	775.93	125.90	7.15E-10	+++++	JAK1	1	Body	65327523	Opensea
cg18515868	1031.99	167.87	7.86E-10	+++++	MAEA	4	Body	1304836	Island
cg09259081	466.78	75.94	7.93E-10	+++++	KIAA1609	16	TSS1500	84538889	Island
cg13009654	1443.53	235.35	8.59E-10	+++++	EGR1	5	Body	137802252	Island
cg20012601	842.76	137.76	9.51E-10	+++++	MAML3	4	Body	141013584	Opensea
cg07133097	-304.06	49.81	1.03E-09	+-----+		2		121497538	Shore
cg27410601	1695.54	277.77	1.03E-09	+++++	TMC6	17	Body	76121564	Opensea
cg20876852	-747.64	122.58	1.07E-09	-----+	MEST	7	5'UTR	130134488	Shore
cg13928490	-486.47	79.80	1.09E-09	+-----+	GUCY1B2	13	TSS200	51640442	Opensea
cg24495681	-1213.65	199.15	1.10E-09	-----	COL14A1	8	Body	121186838	Opensea
cg07732037	499.40	81.97	1.11E-09	+++++	MPHOSPH9	12	TSS1500	123707827	Opensea
cg04521626	-612.49	100.80	1.23E-09	-----+	PLD2	17	Body	4714200	Shelf
cg01877936	-553.34	91.11	1.25E-09	-----+		6		4254352	Shore
cg14242936	555.56	91.49	1.26E-09	+++++	GRASP	12	Body	52404134	Shelf
cg26627956	915.89	150.84	1.26E-09	+++++	CFLAR	2	Body	202004766	Opensea
cg09825327	1114.51	183.77	1.32E-09	+++++	TRAF5	1	5'UTR	211503219	Shelf
cg030515844	-499.22	82.33	1.33E-09	-----+	ZBTB17	1	5'UTR	16277545	Opensea
cg26995690	-520.82	85.90	1.34E-09	+-----+	DCLK1	13	3'UTR	36346376	Opensea
cg12033615	963.55	159.39	1.49E-09	+++++		6		157041546	Shore
cg02283735	-624.17	103.29	1.51E-09	+-----	PTPRF	1	Body	44011587	Opensea
cg19950606	993.47	164.82	1.67E-09	?++++?++-	TMC6	17	Body	76121276	Opensea
cg05135499	417.52	69.29	1.68E-09	+++++		10		21798698	Island
cg10173172	951.92	158.19	1.77E-09	+++?+++++	TMEM65	8	Body	125380812	Shelf
cg16301617	1152.90	191.65	1.79E-09	+++++	TMC8	17	Body	76128906	Shore
cg26995224	623.04	103.73	1.90E-09	+++++	KDM2B	12	Body	121974146	Shore
cg00508575	-702.48	117.00	1.92E-09	-----+	ATP2B1	12	TSS1500	90050967	Opensea
cg20236995	1246.11	207.70	1.98E-09	+++++	PRDM16	1	Body	3071963	Island
cg12160087	858.24	143.10	2.00E-09	+++++	CCDC64	12	3'UTR	120531986	Shelf
cg25311470	937.08	156.34	2.05E-09	+++++	NRCAM	7	5'UTR	107950866	Opensea
cg12801067	-806.99	134.65	2.06E-09	---?-----		12		89380779	Opensea
cg20309640	722.54	120.56	2.06E-09	+++++		1		178027432	Opensea
cg04052013	1204.07	200.98	2.09E-09	-----+	TNNT3	11	Body	1959246	Island
cg12127149	707.25	118.27	2.23E-09	+++++	DYNC1H1	14	Body	102487020	Opensea
cg06937357	1823.94	305.19	2.28E-09	+++++	PACS2	14	Body	105837270	Shore
cg10322443	1137.69	190.52	2.35E-09	+++++		14		65722585	Opensea
cg17942763	990.24	165.94	2.41E-09	+++++	SKI	1	Body	2163472	Shelf
cg05512157	689.94	115.63	2.42E-09	+?+?++++?	DIP2B	12	Body	50901878	Shelf
cg22851200	733.80	123.02	2.45E-09	+++++	TRIP6	7	Body	100465833	Shore
cg26889659	309.01	51.80	2.45E-09	+++++	EXOC2	6	5'UTR	68490	Opensea
cg03180134	721.67	121.07	2.51E-09	+++++	MAML3	4	Body	141013665	Opensea
cg14502713	1188.17	199.58	2.63E-09	+++++	PNPLA7	9	Body	140425395	Opensea
cg19295068	516.99	86.90	2.69E-09	+++++	GLTSCR1	19	3'UTR	48206119	Shore
cg00769843	1550.76	260.88	2.78E-09	+++++	OTUB1	11	Body	63754453	Island
cg04069539	645.58	108.62	2.79E-09	+++++	ACSF3	16	Body	89168963	Shore
cg24013213	-457.03	77.01	2.94E-09	-----+	GUCY1B2	13	Body	51640142	Opensea
cg07807757	-863.40	145.50	2.96E-09	---?+----		7		50894570	Opensea
cg02149069	961.52	162.14	3.03E-09	+++++	IFFO1	12	Body	6649733	Island
cg07285708	1766.48	298.28	3.18E-09	+++++	TATDN2	3	Body	10291324	Shore
cg09888330	1339.68	226.27	3.21E-09	+++++	SH2D2A	1	Body	156786010	Shore
cg19449917	-1059.17	178.93	3.23E-09	---+---+		3		156823356	Opensea
cg03796381	-676.00	114.41	3.45E-09	-----+		15		36650033	Opensea
cg09546258	-1196.09	202.51	3.50E-09	---+-----		11		68444995	Opensea
cg09476997	492.52	83.39	3.51E-09	+++++	SLC9A3R2	16	Body	2087932	Shore
cg05057834	1182.55	200.33	3.57E-09	+++++	ITGA9	3	Body	37495654	Shore
cg09935388	278.44	47.22	3.71E-09	+++++	GFI1	1	Body	92947588	Island
cg17442852	588.09	99.79	3.78E-09	+++++	SLC43A2	17	3'UTR	1478889	Island
cg21350153	920.09	156.17	3.83E-09	+++?+++++	KCNIP3	2	Body	95982371	Opensea

**TABLE 2.** Meta-analysis results with Bonferroni corrected genome-wide significant differential DNA methylation in relation to birth weight on a continuous scale, sorted on p value. *Continued*

CpG	Effect size	SE	P	Direction	Mapped gene	CHR	Location	BP position	Location
cg09026568	703.51	119.55	3.99E-09	+++++	TRABD	22	5'UTR	50625225	Island
cg07624815	1718.03	292.17	4.10E-09	+++++	FGD5	3	Body	14922656	Opensea
cg01693350	-390.43	66.49	4.31E-09	-----	WT1	11	Body	32452187	Island
cg02573176	-589.29	100.47	4.49E-09	-----+	SLC10A5	8	TSS1500	82607505	Opensea
cg24859236	742.62	126.63	4.51E-09	+++	PIK3CD	1	5'UTR	9750213	Island
cg23944298	-711.76	121.40	4.54E-09	---?		3		127248864	Opensea
cg10469774	1008.19	171.96	4.55E-09	+++++		11		44642932	Opensea
cg09790270	-1022.04	174.41	4.63E-09	-----+	SLC41A1	1	Body	205774639	Opensea
cg05433805	-553.08	94.42	4.69E-09	-+?+---	LRRFIP2	3	5'UTR	37204814	Opensea
cg01815912	849.71	145.15	4.79E-09	+++++	NTHL1	16	3'UTR	2089851	Shore
cg04246167	-714.61	122.08	4.81E-09	-----+	C3orf67	3	5'UTR	58984465	Opensea
cg07738730	575.64	98.36	4.84E-09	+++++	IGF2BP1	17	Body	47077165	Shore
cg08743751	771.80	131.94	4.92E-09	+++++	MAST4	5	Body	66462471	Shelf
cg01585860	-885.03	151.41	5.05E-09	-----		6		75922610	Opensea
cg08447200	538.85	92.22	5.13E-09	+++++	PANX2	22	Body	50617460	Island
cg05191655	-933.55	159.80	5.15E-09	---?+---		4		38162793	Opensea
cg27646484	954.09	163.45	5.30E-09	+++++		16		31210736	Shelf
cg15446391	-717.52	123.04	5.48E-09	-----	WT1	11	Body	32452370	Island
cg27292710	851.94	146.15	5.58E-09	+?+++++	KCNJ5	11	5'UTR	128772555	Opensea
cg26340740	-645.10	110.70	5.63E-09	-----	MPEG1	11	1stExon	58980377	Opensea
cg17819250	-649.25	111.60	5.96E-09	-----+		8		36380237	Opensea
cg22039357	-495.61	85.23	6.06E-09	---?-----	C1orf21	1	5'UTR	184388675	Opensea
cg24953596	745.66	128.32	6.21E-09	+++++	MEGF6	1	Body	3515194	Shelf
cg00205605	1116.10	192.11	6.26E-09	+++++		1		2516401	Shore
cg00921266	768.49	132.36	6.40E-09	+++++	HOXA3	7	5'UTR	27153663	Shore
cg21228424	462.71	79.71	6.43E-09	+++++	PRPH2	6	Body	42678314	Opensea
cg14765414	1007.92	173.88	6.77E-09	+++++	MCF2L	13	Body	113652335	Shelf
cg00930606	997.59	172.16	6.85E-09	+?+++++	PATZ1	22	1stExon	31740944	Shore
cg08551036	708.55	122.37	7.04E-09	+++++	ACSF3	16	Body	89219820	Shore
cg21601493	1137.18	196.56	7.24E-09	+++?+++++	IRS2	13	Body	110412178	Opensea
cg08274637	595.96	103.05	7.34E-09	+++++	DLEU7	13	TSS200	51417923	Island
cg07105221	483.27	83.57	7.36E-09	+++++?+	RADIL	7	Body	4859448	Shelf
cg08787968	-421.87	73.03	7.62E-09	-----	WT1	11	Body	32451777	Shore
cg00066351	-646.11	111.93	7.81E-09	-----		7		150130551	Opensea
cg24173182	-470.32	81.50	7.88E-09	-----	HIC1	17	Body	1961286	Island
cg12750675	-667.49	115.68	7.92E-09	---?-----	BRAF	7	Body	140556696	Opensea
cg20494635	914.42	158.48	7.93E-09	+++++	LOC100133545	11	TSS1500	2012071	Shore
cg01066046	1420.32	246.37	8.16E-09	+?+?+++++	SFRS15	21	TSS1500	33105288	Shore
cg06560379	1395.88	242.55	8.66E-09	+++++	NFKBIE	6	Body	44231305	Shore
cg24605046	1073.22	186.49	8.67E-09	+++++	B3GALT4	6	1stExon	33245895	Shore
cg04031093	-596.90	103.75	8.76E-09	-----+		1		233027124	Opensea
cg02449762	566.62	98.56	8.99E-09	+?+?+++++		10		5661741	Opensea
cg19374752	670.33	116.62	9.02E-09	+++++	GRASP	12	Body	52404151	Shelf
cg12036735	-572.56	99.63	9.08E-09	---?+?---		8		20169916	Opensea
cg12876356	226.44	39.43	9.28E-09	+++++	GFI1	1	Body	92946825	Island
cg23147227	1589.11	276.74	9.34E-09	+++++	PTPN6	12	TSS1500	7060187	Opensea
cg20283107	-617.91	107.65	9.48E-09	-----+	FAM91A1	8	Body	124788969	Opensea
cg26709300	623.46	108.70	9.71E-09	+++++	YPEL3	16	1stExon	30106682	Shore
cg07638500	847.35	147.90	1.01E-08	+++++	MYLK	3	Body	123371420	Opensea
cg25939371	-666.84	116.40	1.01E-08	+?+?-----	BRUNOL6	15	Body	72598286	Opensea
cg02355304	614.98	107.44	1.04E-08	+++++	MIR589	7	TSS1500	5536984	Shelf
cg25311963	717.11	125.31	1.05E-08	+?+?+++++		1		1546691	Shelf
cg24020157	-705.03	123.21	1.05E-08	---?-----	RASGEF1A	10	Body	43697521	Shore
cg20663495	870.12	152.13	1.07E-08	+?+?+++++	B3GNTL1	17	Body	80943851	Opensea
cg04315923	-524.89	91.83	1.09E-08	---?+?---		16		29634191	Opensea
cg05208050	-1226.90	214.69	1.10E-08	---+?+---	MOGS	2	Body	74690526	Shore
cg20102045	511.15	89.47	1.11E-08	+++++	MYO16	13	Body	109663681	Opensea
cg03747456	-658.90	115.37	1.12E-08	-----+	KRT80	12	TSS1500	52586127	Opensea
cg11125758	525.38	92.02	1.13E-08	+++++		14		83591622	Opensea
cg03543319	-850.23	148.97	1.15E-08	-----	PROKR1	2	Body	68882249	Shore
cg14731462	-700.86	122.82	1.15E-08	-----+	PTPRE	10	5'UTR	129789429	Opensea
cg15206445	870.58	152.56	1.15E-08	+++++	SPG7	16	TSS1500	89573312	Shore
cg26894079	-714.30	125.18	1.16E-08	---+?+---	ASAM	11	Body	122954435	Opensea

4

**TABLE 2.** Meta-analysis results with Bonferroni corrected genome-wide significant differential DNA methylation in relation to birth weight on a continuous scale, sorted on p value. *Continued*

CpG	Effect size	SE	P	Direction	Mapped gene	CHR	Location	BP position	Location
cg05119988	-496.03	87.02	1.20E-08	-----+--	SC4MOL	4	5'UTR	166251189	Shelf
cg02583484	933.54	163.79	1.20E-08	-----+++++	HNRNPA1	12	Body	54677008	Shelf
cg05478824	1328.72	233.21	1.22E-08	+++++++	ASPSR1	17	Body	79970135	Shelf
cg21249754	1035.03	181.81	1.25E-08	+++++++	PTPN6	12	TSS1500	7060206	Opensea
cg12419863	-713.99	125.42	1.25E-08	-----	PLAGL1	6	Body	144263450	Opensea
cg06500792	-720.87	126.66	1.26E-08	-----+		2		65006618	Opensea
cg03144619	377.27	66.36	1.31E-08	+++++++	GALNT2	1	3'UTR	230415668	Island
cg06362282	945.73	166.46	1.34E-08	++-+++++	B3GALT4	6	1stExon	33245893	Shore
cg12192749	-755.18	132.97	1.35E-08	-----+		7		17029795	Opensea
cg21631918	915.07	161.13	1.36E-08	+++++++	STRA6	15	TSS200	74501549	Opensea
cg23317857	756.83	133.37	1.39E-08	+++?+++++	TFEB	6	5'UTR	41696297	Opensea
cg23404711	651.54	114.90	1.43E-08	+++++++	FOS	14	Body	75747047	Shore
cg06685437	740.61	130.69	1.45E-08	+++++++	RAI1	17	5'UTR	17669973	Opensea
cg11474701	-556.86	98.28	1.46E-08	---?+--+		11		121193898	Opensea
cg01619846	1044.71	184.43	1.48E-08	+++++++	UHRF1	19	Body	4911475	Shore
cg04830471	-1245.34	220.13	1.54E-08	---?+---	CCDC155	19	TSS1500	49890365	Shore
cg21788755	795.38	140.59	1.54E-08	+++++++	TREX1	3	Body	48508391	Opensea
cg26884581	881.30	155.87	1.57E-08	+++++++	PYGM	11	1stExon	64527414	Opensea
cg08756594	1089.51	192.76	1.59E-08	+++++++	LSP1	11	5'UTR	1892392	Island
cg06405563	638.30	112.95	1.59E-08	?++?+++++		17		73577744	Opensea
cg11036672	867.44	153.50	1.60E-08	+++++++	C4orf23	4	Body	8476435	Opensea
cg00119811	-1082.38	191.57	1.60E-08	---+-----	RGNEF	5	Body	73092353	Opensea
cg08426200	-759.54	134.47	1.62E-08	-----+	AGPHD1	15	5'UTR	78802280	Shore
cg03565674	1036.58	183.59	1.64E-08	+++++-----	HCCA2	11	Body	1507321	Opensea
cg18050903	-1234.58	218.87	1.69E-08	-----+--	RASGEF1B	4	Body	82375718	Opensea
cg00091302	-572.39	101.49	1.70E-08	-----+		1		182602986	Opensea
cg15490335	971.07	172.20	1.71E-08	+++++++	CASZ1	1	Body	10723174	Shelf
cg12547807	-1065.07	189.05	1.76E-08	-----+		1		9473751	Opensea
cg24304210	-595.31	105.68	1.77E-08	-----+--	PAK1	11	5'UTR	77118761	Shelf
cg05901196	714.09	126.78	1.78E-08	++-+++++	CCDC6	10	Body	61664954	Shore
cg10670376	-1556.44	276.41	1.79E-08	---?-----	ARHGAP26	5	Body	142432538	Opensea
cg23661483	801.25	142.30	1.80E-08	+++++++	ILVBL	19	Body	15227219	Shelf
cg04510459	-857.63	152.36	1.81E-08	-----+--		1		92048287	Opensea
cg20204986	-502.39	89.33	1.86E-08	-----+	WT1	11	Body	32448067	Shore
cg06850687	-761.17	135.35	1.87E-08	-----+--		12		56587526	Shelf
cg04304130	668.51	118.94	1.90E-08	+++?+++++	LOC221710	6	Body	11111894	Opensea
cg24162603	-1015.35	180.69	1.92E-08	---+-----?	EEFSEC	3	Body	127945858	Opensea
cg10735015	-411.54	73.24	1.92E-08	-----+--?	PHACTR2	6	TSS1500	143928643	Opensea
cg24480146	-854.36	152.20	1.98E-08	-----?+--		10		43430536	Shore
cg06287775	543.60	96.86	2.00E-08	+++?++-+++	FOXK1	7	Body	4784174	Shore
cg10421188	-790.72	140.92	2.01E-08	-----	CREB5	7	Body	28824953	Opensea
cg09098522	802.35	143.01	2.02E-08	++-+++++		10		31435673	Opensea
cg06072822	1217.68	217.04	2.02E-08	+++?+++++	MCF2L	13	Body	113652069	Shelf
cg14827643	733.85	130.81	2.02E-08	+++++++	RGS12	4	Body	3365442	Shore
cg02988730	840.61	149.93	2.06E-08	+++++++	AMPD2	1	Body	110167509	Opensea
cg27389255	657.07	117.20	2.07E-08	+++++++	PDE3B	11	TSS1500	14664598	Shore
cg02704502	560.32	99.95	2.07E-08	+++++++		11		2036580	Shelf
cg05247391	1550.83	277.00	2.16E-08	+++++++	XKR9	8	5'UTR	71581649	Island
cg22242842	-698.59	124.78	2.16E-08	-----		2		85167149	Opensea
cg01832549	-551.21	98.53	2.22E-08	-----+--	CAPZB	1	Body	19774989	Opensea
cg06612130	-689.54	123.27	2.22E-08	+-----+		4		185269299	Opensea
cg25383605	854.52	152.82	2.25E-08	+++++++	TIAM2	6	Body	155538047	Shore
cg01731783	1013.13	181.35	2.32E-08	+++++-----	C14orf43	14	5'UTR	74211788	Opensea
cg19631472	928.66	166.25	2.32E-08	+++++++	LDHA	11	TSS1500	18415641	Shore
cg14160422	657.71	117.76	2.33E-08	+++?+++++	SLC44A2	19	TSS1500	10735548	Shore
cg00496272	1567.21	280.60	2.33E-08	+++++++	CENPT	16	5'UTR	67877232	Shore
cg18316974	469.85	84.17	2.37E-08	+++++++?+	GFI1	1	Body	92947035	Island
cg04086394	1496.94	268.21	2.39E-08	+++++-----		1		193082764	Opensea
cg23529896	-556.46	99.77	2.44E-08	---+-----		2		182247492	Opensea
cg22057874	-595.55	106.84	2.48E-08	-----+--	OSBPL6	2	TSS200	179184866	Opensea
cg02462416	460.43	82.61	2.50E-08	+++++++		11		2036573	Shelf
cg09063663	1054.66	189.27	2.51E-08	+++++++		1		16484811	Shelf
cg17924072	-639.34	114.75	2.53E-08	---?-----+	HMHA1	19	Body	1075067	Island



**TABLE 2.** Meta-analysis results with Bonferroni corrected genome-wide significant differential DNA methylation in relation to birth weight on a continuous scale, sorted on p value. *Continued*

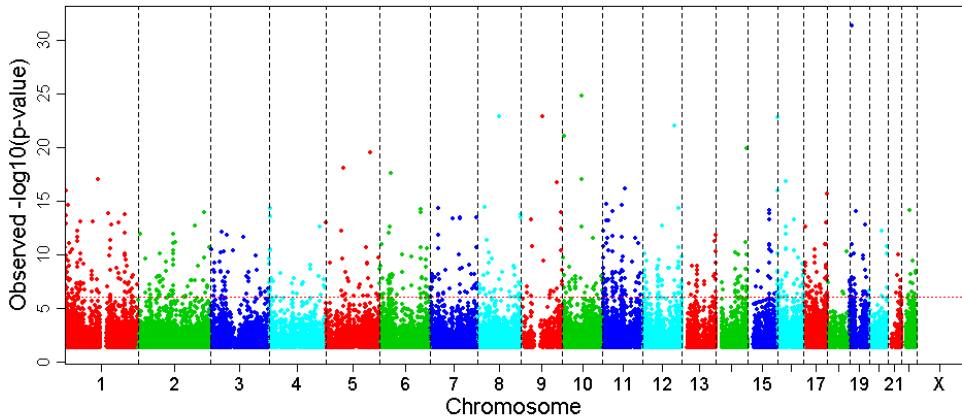
CpG	Effect size	SE	P	Direction	Mapped gene	CHR	Location	BP position	Location
cg08818716	1133.00	203.38	2.53E-08	+++++++	RADIL	7	Body	4876909	Shore
cg10780778	-626.39	112.45	2.54E-08	---?----+		6		26351915	Opensea
cg03395188	876.45	157.35	2.55E-08	?+++++++	CTDSP1	2	Body	219266059	Shore
cg10583311	691.84	124.33	2.63E-08	+++++++	FBRS1	12	Body	133082545	Opensea
cg03861784	-669.99	120.44	2.65E-08	---?+----		12		66442412	Opensea
cg24279744	783.79	140.92	2.67E-08	+++++++	ATP2B2	3	Body	10408402	Opensea
cg07452706	1157.02	208.10	2.70E-08	+++++++	NUDT14	14	TSS1500	105649097	Shore
cg03661409	-1126.31	202.60	2.71E-08	----+?+-	GLS	2	Body	191812319	Opensea
cg01400685	-630.92	113.51	2.73E-08	-----	FADS2	11	Body	61598025	Shore
cg24764861	638.36	114.88	2.75E-08	+++++++	CCDC154	16	TSS1500	1495122	Island
cg09255514	-552.24	99.46	2.81E-08	----+?+-	ABCC1	16	Body	16181832	Opensea
cg12949547	-975.91	175.86	2.87E-08	----+?+-		12		58718950	Opensea
cg25306480	681.94	122.91	2.88E-08	+++++++	PWWP2B	10	3'UTR	134226517	Opensea
cg00780426	-672.96	121.30	2.89E-08	---?-----		6		27838209	Shore
cg13725803	-499.86	90.11	2.91E-08	+---+?+-	GUCY1B2	13	TSS200	51640297	Opensea
cg24727480	837.18	150.97	2.93E-08	+++++++	KCTD10	12	Body	109901083	Opensea
cg07466788	1323.22	238.77	2.99E-08	+++++++	SLC16A3	17	Body	80194552	Island
cg02370100	1171.10	211.34	3.00E-08	+++++++	ABCG1	21	Body	43655256	Island
cg05673882	-445.00	80.36	3.06E-08	----+?+-	POLK	5	Body	74862702	Opensea
cg18489994	-893.94	161.49	3.10E-08	+-----		4		15235419	Opensea
cg13937905	971.66	175.61	3.15E-08	+++++++	RARG	12	Body	53612551	Shore
cg01099825	653.22	118.10	3.19E-08	+++++++	COBRA1	9	Body	140166114	Shore
cg04500896	757.90	137.08	3.22E-08	+++++++	LOXL3	2	Body	74775717	Shore
cg13638420	-622.87	112.66	3.23E-08	-----+	WT1	11	Body	32448293	Island
cg06128598	-906.45	164.05	3.29E-08	?-?-?-?-?	PYY2	17	TSS1500	26552870	Shore
cg02264407	639.82	115.86	3.34E-08	+++++++	LMO7	13	Body	76430665	Opensea
cg01741041	1177.52	213.29	3.37E-08	+++++++	ACTB	7	Body	5567862	Shore
cg17828445	1312.74	237.80	3.38E-08	+?++++?+	MARCH9	12	Body	58150842	Shore
cg19921353	-1011.52	183.40	3.48E-08	-----	HLA-DBP1	6	TSS200	33043574	Opensea
cg06945690	867.78	157.43	3.54E-08	?++++?+++?	ZNF167	3	Body	44621374	Shore
cg08972588	-913.98	165.85	3.57E-08	----+?+-	TNKB	6	Body	32014674	Opensea
cg11077681	984.85	178.72	3.58E-08	+++++++	MRV1	11	1stExon	10715188	Opensea
cg05859076	628.55	114.23	3.74E-08	+?+++++	RAB43	3	Body	128838350	Shore
cg08970648	651.02	118.35	3.78E-08	+++++++	CISH	3	3'UTR	50644192	Opensea
cg17783244	535.87	97.53	3.93E-08	+++++++	PATZ1	22	Body	31738549	Shelf
cg21768858	-675.61	123.07	4.03E-08	-----+	DHCR24	1	Body	55351659	Shore
cg07795325	-725.39	132.15	4.04E-08	-----+	CAMK2D	4	Body	114557667	Opensea
cg19876649	-643.65	117.35	4.14E-08	----+?+-	MYOM1	18	TSS200	3220273	Opensea
cg06826457	697.07	127.10	4.14E-08	+++++++		12		12867669	Shelf
cg07810039	-481.91	87.90	4.20E-08	----+?+-	TGFB2	1	Body	218524558	Opensea
cg24973755	716.91	130.81	4.24E-08	+++++++	MAEA	4	Body	1304972	Island
cg02753354	-934.45	170.69	4.39E-08	-----+	HMHA1	19	Body	1074727	Island
cg05910124	722.18	131.93	4.40E-08	+++++++	TRAPPC9	8	Body	141057427	Island
cg12061886	888.03	162.25	4.42E-08	+++++++	FOS	14	Body	75746793	Island
cg08420923	611.01	111.64	4.43E-08	+++++++	ANKRD11	16	5'UTR	89436447	Shelf
cg02332696	-1479.46	270.36	4.44E-08	-----		10		21783091	Shore
cg20671781	811.24	148.33	4.52E-08	+++++++		10		43626471	Opensea
cg11067407	-674.46	123.33	4.53E-08	-----		11		113599852	Opensea
cg25741837	704.29	128.83	4.59E-08	+++++++	SMYD5	2	Body	73452813	Opensea
cg14177925	-765.29	140.02	4.61E-08	---?-----		14		22465676	Opensea
cg10950251	-587.69	107.54	4.63E-08	---?-----		1		204466432	Shelf
cg00575744	-766.49	140.47	4.85E-08	-----	HLA-DMB	6	1stExon	32908642	Opensea
cg01858866	891.33	163.47	4.96E-08	+++++++	PABPC4	1	Body	40040016	Shore
cg08381620	-275.81	50.60	5.02E-08	----+?+-		13		24062873	Opensea
cg03918288	1092.96	200.61	5.09E-08	+++++++	SPATC1	8	Body	145101324	Shore
cg00233028	740.70	135.97	5.11E-08	+++++++		11		44642914	Opensea
cg19320261	1149.49	211.09	5.17E-08	+++++++	ROR1	1	Body	64409138	Opensea
cg19358373	771.15	141.62	5.18E-08	+++++++		12		133414571	Shore
cg24332710	700.38	128.69	5.26E-08	+?+++++	AKR1A1	1	TSS1500	46016173	Shore
cg16234490	-682.06	125.34	5.28E-08	----+?+-		4		77138082	Shelf
cg01115380	-510.76	93.95	5.44E-08	---?+----		6		1552904	Shelf
cg09109520	933.55	171.72	5.44E-08	+++++++	GPR56	16	5'UTR	57673258	Opensea
cg00831931	-1062.25	195.40	5.44E-08	----+?+-	TCF7L2	10	Body	114823094	Opensea

4

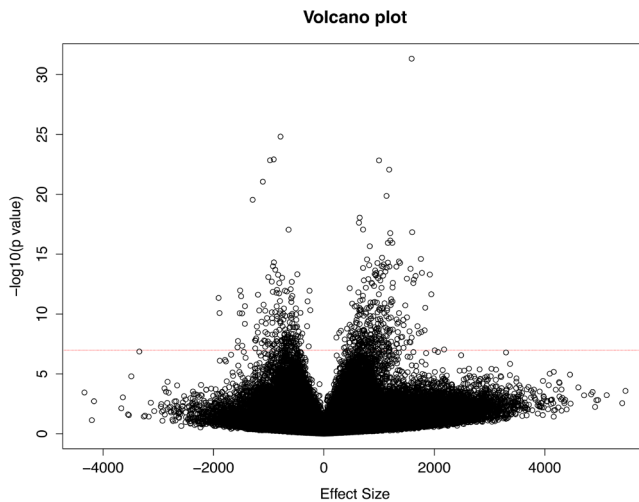
**TABLE 2.** Meta-analysis results with Bonferroni corrected genome-wide significant differential DNA methylation in relation to birth weight on a continuous scale, sorted on p value. *Continued*

CpG	Effect size	SE	P	Direction	Mapped gene	CHR	Location	BP position	Location
cg02431260	937.82	172.55	5.48E-08	+++++-----	ESAM	11	Body	124628888	Island
cg15959363	-535.73	98.58	5.49E-08	----?-----?	C4orf32	4	Body	113091339	Opensea
cg02855778	770.48	141.78	5.50E-08	+++++-----	PTPRN2	7	Body	157462914	Opensea
cg21649604	1047.32	192.78	5.55E-08	-----+-----	RPL3	22	Body	39714288	Shore
cg14909179	389.96	71.86	5.73E-08	+?+-----	CHID1	11	Body	882687	Shore
cg25110523	806.76	148.73	5.82E-08	?+-----	ZBTB12	6	Body	31868025	Shore
cg23960707	848.97	156.75	6.09E-08	+++++-----	SFRS1	17	Body	56083289	Shore
cg21193744	-1565.27	289.01	6.10E-08	---?-----	ANGPT2	8	Body	6405105	Opensea
cg13993914	863.20	159.39	6.11E-08	+++++-----	COBRA1	9	Body	140165710	Shore
cg15822394	846.88	156.38	6.12E-08	+++++-----	FCH01	19	Body	17877733	Island
cg08530838	1171.67	216.46	6.21E-08	+++++-----		5		114193858	Opensea
cg25199005	-728.80	134.69	6.26E-08	-----+-----	SMYD3	1	Body	246446300	Opensea
cg26596307	1171.69	216.58	6.31E-08	+++++-----	CX3C1	16	Body	57411505	Opensea
cg20386404	-679.87	125.74	6.41E-08	---+-----	PTPN14	1	Body	214577721	Opensea
cg08277369	364.55	67.48	6.59E-08	+++++-----	KIAA1609	16	TSS1500	84538885	Island
cg20784591	1442.62	267.05	6.59E-08	-----+-----	PILRA	7	Body	99972461	Opensea
cg09915396	-767.03	142.00	6.61E-08	-----+-----	RAP1GAP2	17	Body	2907895	Opensea
cg00971695	-727.80	134.82	6.73E-08	-----+-----		14		65169664	Shore
cg01490733	1355.48	251.11	6.74E-08	+++++-----		1		2247066	Island
cg08202399	1184.26	219.73	7.06E-08	+++++-----	MKL2	16	Body	14280512	Opensea
cg18702935	-855.88	158.86	7.14E-08	+--+-----		13		48507440	Opensea
cg26750893	-531.95	98.74	7.15E-08	+-----		2		38043481	Opensea
cg02397552	606.81	112.64	7.17E-08	+++?+-----	CLN5	13	3'UTR	77576078	Opensea
cg00382138	-504.36	93.64	7.19E-08	---?+-----?	CFI	4	1stExon	110723299	Opensea
cg03444940	565.51	105.02	7.26E-08	+++?+-----	TSC2	16	Body	2133637	Shore
cg00654448	-1243.33	230.93	7.29E-08	---+-----		8		142365315	Shore
cg21822187	-528.04	98.12	7.38E-08	-----+--	HMGGA2	12	Body	66286141	Opensea
cg18642234	863.18	160.41	7.40E-08	+++++-----	GPX1	3	3'UTR	49394622	Shore
cg02640173	-495.92	92.18	7.46E-08	+-----+?	GLI2	2	Body	121605707	Opensea
cg00443981	570.40	106.05	7.51E-08	+++++-----	C17orf64	17	TSS200	58499679	Shore
cg25921609	488.44	90.91	7.75E-08	+++++-----	MYH10	17	Body	8379225	Shore
cg04276057	983.73	183.19	7.88E-08	+++++-----	C2orf82	2	Body	233736931	Shelf
cg16855355	-707.52	131.82	7.99E-08	+-----+?		4		185269126	Opensea
cg03278811	1123.27	209.41	8.14E-08	+++++-----	FOXO3	6	Body	108883330	Island
cg06996175	749.08	139.95	8.67E-08	+++++-----	GNG7	19	5'UTR	2546877	Island
cg00533407	-645.29	120.57	8.71E-08	-----	ACCN3	7	TSS1500	150745278	Shore
cg04884025	-815.84	152.63	9.03E-08	---+-----	ZFYVE9	1	Body	52704337	Opensea
cg21586223	-508.08	95.08	9.11E-08	---?+-----		13		49227121	Opensea
cg26357744	2176.87	407.41	9.13E-08	+-----+--	RASSF1	3	Body	50374304	Island
cg07814318	651.09	121.96	9.37E-08	+++++-----	KLF13	15	Body	31624584	Shelf
cg22479226	893.79	167.44	9.40E-08	+++++-----	LARP1	5	Body	154153028	Opensea
cg10059056	1190.85	223.22	9.56E-08	+++++-----	DGKZ	11	Body	46355283	Shore
cg16875057	-646.89	121.32	9.72E-08	---?+-----	STK39	2	Body	169006003	Opensea
cg27066989	-668.50	125.47	9.93E-08	-----+--	GUCY1B2	13	TSS200	51640362	Opensea
cg13131501	995.44	186.93	1.01E-07	+++++-----	RBM8A	1	Body	145508262	Shore
cg02461269	-901.68	169.48	1.04E-07	---?+-----		6		156509874	Opensea
cg03348792	610.77	114.82	1.04E-07	+-----+--	KRT1	12	TSS1500	53075482	Opensea
cg03174507	880.63	165.55	1.04E-07	+++++-----		10		21789582	Island

\*Meta-analysis results of the association between DNA methylation in newborn cord blood and birth weight, adjusted for covariates. Selected genome-wide significant CpGs after Bonferroni correction. Results sorted by the p-values of associations with birth weight. Column headers: CpG: Cytosine-phosphate-Guanine site; Effect size: regression coefficient; SE: standard error; P: P-value; Direction: Direction of effect across cohorts included in the statistical model: maternal smoking during pregnancy associated with increased (+) or decreased (-) methylation, or missing result (?), in alphabetical order of cohorts; Mapped Gene: UCSC annotated gene; CHR: chromosome; Location in gene: UCSC gene region feature category; BP position: basepair position; Location: CpG islands/shores/shelves/open sea.



**FIGURE 1.** Manhattan plot for chromosomal location of the significant CpGs for the meta-analysis on cord blood DNA methylation and birth weight, the red dashed line shows the Bonferroni significance threshold ( $P < 1.055 \cdot 10^{-7}$ ).



**FIGURE 2.** Volcano plot indicating CpG-specific directions of effect and corresponding p-values for the meta-analysis of the association between cord blood DNA methylation and birth weight, the red line shows the Bonferroni significance threshold ( $P < 1.055 \cdot 10^{-7}$ ).

## DISCUSSION

In this meta-analysis of twelve EWASs in 6,027 newborns we found 490 CpG sites in 293 genes of which DNA methylation levels were associated with birth weight. Additionally, we found 58 CpGs in 36 genes to be differentially methylated in high birth weight newborns (>4000 g) versus normal birth weight newborns (2500-4000 g).

The *WNT1*, *LOXL1* and *PRDM16* genes had the largest number of significant CpGs with 8, 7 and 5 CpGs mapping to these genes, respectively. The wingless-type MMTV integration site family, member 1 (*WNT1*) gene has been implicated in developmental processes, e.g. embryogenesis. This gene is part of the *Wnt family* which is involved in many features of embryonic development<sup>25,26</sup>. The Wnt signaling pathway has previously also been linked to adipogenesis, insulin resistance, bone fragility and several types of cancer<sup>26-28</sup>. The lysyl oxidase-like 1 (*LOXL1*) gene is essential for biogenesis of connective tissue. Furthermore, it is associated with developmental regulation and cell growth control<sup>29</sup>. The *lysyl oxidase family* has also been linked to the development of various organs, e.g. the cardiovascular and respiratory systems<sup>30,31</sup>. From the PR domain containing 16 (*PRDM16*) gene we know that it is associated with brown fat development<sup>32</sup>. Brown fat is an active type of adipose tissue in inflammatory state that could counteract insulin resistance and the development of obesity<sup>32,33</sup>. Overall, we can link these three genes to embryonic development and adipogenesis. Therefore, it makes sense that these genes were found in this meta-EWAS for birth weight.

Only 15 of the 490 CpGs identified in our study have been found before in an EWAS for methylation and birth weight conducted in the MoBa1 cohort and mapping to seven genes: *UHRF1*, *ARID5B*, *XRCC3*, *ANKRD11*, *KLF9*, *MFS10* and *PEBP4*<sup>9</sup>. Of those 15 CpGs, 10 are in our top 30 most significant findings, which is not surprising because the same MoBa1 cohort is the largest studies in our meta-EWAS. Interestingly, in a previous EWAS in our PACE consortium, 80 of the 293 genes in our meta-EWAS were associated with maternal smoking (Chapter 3 in this thesis). Moreover, we previously found methylation levels of three CpGs in the *GFI1* gene to be affected by maternal smoking, which mediated 20% of the association between smoking and offspring birth weight<sup>11</sup>. In this meta-EWAS on birth weight, we confirmed two (cg09935388 and cg12876356) of those three CpG sites. This could indicate that DNA methylation plays a role in the underlying biological mechanism linking adverse intrauterine exposures to health outcomes, e.g. birth weight. Overall, we can conclude that these eight "known" genes all have a logical link towards birth weight and foetal development, as they have functions in embryonic development, cell growth, adipogenesis and lipid storage<sup>34-37</sup>.

Our meta-EWAS also identified a gene that was previously found in a meta-analysis of genome-wide association studies (meta-GWAS) for birth weight: *HMG2*<sup>38</sup>. *HMG2* was also linked to height at birth, during childhood and adulthood in two other meta-GWASs<sup>39,40</sup>. Additionally, the Insulin-like Growth Factor (*IGF*) family has previously also

been related to fetal growth and birth weight in GWAS and in a recent candidate-gene methylation study<sup>35,41</sup>. Another candidate-gene study also identified the pleckstrin homology-like domain, family A, member 2 (*PHLDA2*) gene associated with birth weight similar as in our meta-EWAS finding<sup>42</sup>.

Interestingly, our current meta-EWAS resulted in many more significant signals pointing to candidate genes compared to previous meta-GWASs. For example, a meta-GWAS in 2010 of 10,623 individuals in the discovery phase resulted in only three genes (*LEKR1*, *CCNL1* and *ADCY5*) to be associated with birth weight<sup>43</sup>. Another more recent meta-GWAS in 26,836 individuals resulted in four more genes associated to birth weight: *CCNL1* and *ADCY5* were confirmed and *HMGA2*, *CDKAL1*, *LCORL* and *ADRB1* (and *5q11.2*) were novel genes<sup>38</sup>. In comparison, our meta-EWAS consisted of a much smaller sample size (n=6,027) and resulted in many more genes (293 genes) related to birth weight. One potential reason for this larger number of genes in meta-EWAS might be that differential methylation signals as assessed by the meta-EWAS reflect a multitude of subtle changes that are affected by environmental exposures. Another potential reason might be that methylation is measured on a quantitative scale between zero and one, and single nucleotide polymorphisms (SNPs) are measured on an ordinal scale (0, 1 or 2 risk alleles). Our current study confirmed the interesting added value of EWAS for association with birth weight, by adding many genes to the known list of genes associated with birth weight. Because the number of significant genes in EWAS is much larger than in GWAS, the variance in birth weight explained by meta-EWAS could be higher than in meta-GWAS, even in a much smaller sample size. This would suggest that increasing sample sizes in meta-EWAS may facilitate to explain even more variance in birth weight, as was recently also described for the association with overweight<sup>44</sup>. Subsequently, these differentially methylated genes could be seen as a proxy for the combined effect of intrauterine exposures, which might be used for prediction of metabolic outcomes in the context of the DOHaD hypothesis. In that case, the methylation risk score may be combined with a genetic risk score, as Shah and colleagues did for BMI, to examine further improvement of prediction of birth weight related health outcomes<sup>44</sup>. "The epigenetic clock" by Horvath is a good example of such a methylation risk score that has been used to predict ageing<sup>45</sup>. Comparable risk scores have previously also been developed based on GWAS results<sup>46,47</sup>.

To our knowledge this is the first meta-EWAS on the association between cord blood methylation and birth weight. The combination of multiple birth cohorts from the US and

Europe resulted in a large sample size, which increased the power of our analyses and improved the generalizability of our results. Using this meta-EWAS approach we found that differential methylation of multiple novel genes was associated with birth weight. Only 11 genes have previously been identified in association with birth weight in EWASs or GWASs. Therefore, this meta-EWAS adds a large amount of knowledge (279 novel genes) to the current understanding of biological pathways associated with intrauterine growth and birth weight. This new knowledge could ideally add to the prediction of health outcomes in later life, e.g. cardiometabolic problems, that are known to be associated with birth weight<sup>5</sup>. However, it should be noted that the direction of causation could not be established in this study.

One potential source of bias in EWASs might be caused by (extreme) outlying methylation levels as a source of false positive findings. For example, if an individual in our study had a very high birth weight and also an extremely low methylation level for a specific CpG site, the results of that particular CpG site may be completely driven by this one individual. To remove this potential bias by influential extreme outliers, all cohorts in our meta-EWAS trimmed the methylation set using: (25<sup>th</sup> percentile - 3\*IQR) and (75<sup>th</sup> percentile + 3\*IQR), where IQR = interquartile range. Thus for each probe values outside this range were set to NA. We performed a meta-EWAS before and after this trim and found 680 Bonferroni significant CpGs before this exclusion of outliers. Thus, with this trim we excluded 190 CpGs (28% of all 680 CpGs) that probably were false positive findings.

Another potential limitation of our study is that it was cross-sectional, because both cord blood DNA methylation and birth weight were measured simultaneously, immediately after birth. Consequently, we cannot make a clear distinction between causality and reverse causation, but we intend to replicate our analyses in an adult cohort to examine whether our significant CpGs related to birth weight are stable, i.e., whether they remain differentially methylated over time.

At any rate, cord blood is a great resource for EWASs on birth weight, because it has not yet been influenced by (adverse) exposures throughout life. Therefore, we can exclude the possibility of such confounding. Still, the use of (cord) blood in EWASs is a topic of debate, because it consists of a mixture of cell types. However, we obviated this potential issue by estimating proportions of six cell types (CD8+ T cells, CD4+ T cells, NK cells, B cells, granulocytes and monocytes) in each cohort and correcting the regression analyses for these proportions, using the well-known method introduced by Houseman

and colleagues and the corresponding Reinius dataset<sup>22,23</sup>. Additionally, we should take into account that cord blood can more easily be collected in large birth cohorts compared to other tissues that might be more specifically related to birth weight (e.g. fat or muscle tissue). Collecting these other tissues in newborns might not be ethically feasible, thus cord blood has been chosen in the birth cohorts in our consortium. Unfortunately, we did not have gene expression data available in this meta-analysis at the time of submission of this thesis. However, we are planning gene expression analyses to confirm that our differential methylation signals also translate into differential gene expression.

It should be taken into account that in our study we excluded preterm newborns, which also resulted in exclusion of most of the (very) low birth weight newborns. Therefore we could not draw any conclusions about low birth weight, because the sample size was too small for this analysis. We chose to exclude preterm births because we assume that these newborns were exposed to other intrauterine influences, e.g. pre-eclampsia, maternal smoking and placental damage<sup>48</sup>. These intrauterine and placental disturbances might be more strongly associated with both birth weight and DNA methylation<sup>49</sup>.

## CONCLUSION

This first meta-analysis of multiple EWASs for birth weight yielded a steep increase in the number of genes showing differential methylation associated with birth weight. This large list of differentially methylated genes may provide insight into the biological mechanisms underlying the relation between intrauterine exposures and adult health.

## REFERENCES

- 1 Aagaard-Tillery KM, Porter TF, Lane RH, Varnier MW, Lacoursiere DY. In utero tobacco exposure is associated with modified effects of maternal factors on fetal growth. *Am J Obstet Gynecol* 2008; 198: 66 e1-6.
- 2 HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, et al. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008; 358: 1991-2002.
- 3 Wadhwa PD, Sandman CA, Porto M, Dunkel-Schetter C, Garite TJ. The association between prenatal stress and infant birth weight and gestational age at birth: a prospective investigation. *Am J Obstet Gynecol* 1993; 169: 858-65.
- 4 Barker DJ. The fetal origins of hypertension. *J Hypertens Suppl* 1996; 14: S117-20.
- 5 Barker DJ, Osmond C, Forsen TJ, Kajantie E, Eriksson JG. Trajectories of growth among children who have coronary events as adults. *N Engl J Med* 2005; 353: 1802-9.
- 6 Kupers LK, L'Abee C, Bocca G, Stolk RP, Sauer PJ, Corpeleijn E. Determinants of Weight Gain during the First Two Years of Life-The GECKO Drenthe Birth Cohort. *PLoS One* 2015; 10: e0133326.
- 7 Robinson S, Yardy K, Carter V. A narrative literature review of the development of obesity in infancy and childhood. *J Child Health Care* 2012; 16: 339-54.
- 8 Breton CV, Siegmund KD, Joubert BR, et al. Prenatal tobacco smoke exposure is associated with childhood DNA CpG methylation. *PLoS One* 2014; 9: e99716.
- 9 Engel SM, Joubert BR, Wu MC, et al. Neonatal genome-wide methylation patterns in relation to birth weight in the Norwegian Mother and Child Cohort. *Am J Epidemiol* 2014; 179: 834-42.
- 10 Joubert BR, Haberg SE, Nilsen RM, et al. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ Health Perspect* 2012; 120: 1425-31.
- 11 Kupers LK, Xu X, Jankipersadsing SA, et al. DNA methylation mediates the effect of maternal smoking during pregnancy on birthweight of the offspring. *Int J Epidemiol* 2015;.
- 12 Markunas CA, Xu Z, Harlid S, et al. Identification of DNA Methylation Changes in Newborns Related to Maternal Smoking during Pregnancy. *Environ Health Perspect* 2014;.
- 13 Suter M, Ma J, Harris A, et al. Maternal tobacco use modestly alters correlated epigenome-wide placental DNA methylation and gene expression. *Epigenetics* 2011; 6: 1284-94.
- 14 Azzi S, Sas TC, Koudou Y, et al. Degree of methylation of ZAC1 (PLAGL1) is associated with prenatal and post-natal growth in healthy infants of the EDEN mother child cohort. *Epigenetics* 2014; 9: 338-45.
- 15 Bouwland-Both MI, van Mil NH, Stolk L, et al. DNA methylation of IGF2DMR and H19 is associated with fetal and infant growth: the generation R study. *PLoS One* 2013; 8: e81731.
- 16 Cheong CY, Chng K, Lim MK, et al. Alterations to DNA methylation and expression of CXCL14 are associated with suboptimal birth outcomes. *J Hum Genet* 2014; 59: 504-11.
- 17 Haworth KE, Farrell WE, Emes RD, et al. Methylation of the FGFR2 gene is associated with high birth weight centile in humans. *Epigenomics* 2014; 6: 477-91.
- 18 Adkins RM, Tylavsky FA, Krushkal J. Newborn umbilical cord blood DNA methylation and gene expression levels exhibit limited association with birth weight. *Chem Biodivers* 2012; 9: 888-99.
- 19 Turan N, Ghalwash MF, Katari S, Coutifaris C, Obradovic Z, Sapienza C. DNA methylation differences at growth related genes correlate with birth weight: a molecular signature linked to developmental origins of adult disease? *BMC Med Genomics* 2012; 5: 10.8794-5-10.
- 20 Tan Q, Frost M, Heijmans BT, et al. Epigenetic signature of birth weight discordance in adult twins. *BMC Genomics* 2014; 15: 1062.2164-15-1062.
- 21 Souren NY, Lutsik P, Gasparoni G, et al. Adult monozygotic twins discordant for intra-uterine growth have indistinguishable genome-wide DNA methylation profiles. *Genome Biol* 2013; 14: R44.2013-14-5-r44.
- 22 Houseman EA, Accomando WP, Koestler DC, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics* 2012; 13: 86.2105-13-86.
- 23 Reinius LE, Acevedo N, Joerink M, et al. Differential DNA methylation in purified human blood cells: implications for cell lineage and studies on disease susceptibility. *PLoS One* 2012; 7: e41361.



- 24 Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010; 26: 2190-1.
- 25 Gavin BJ, McMahon JA, McMahon AP. Expression of multiple novel Wnt-1/int-1-related genes during fetal and adult mouse development. *Genes Dev* 1990; 4: 2319-32.
- 26 Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell* 2006; 127: 469-80.
- 27 Yang X, Jansson PA, Nagaev I, et al. Evidence of impaired adipogenesis in insulin resistance. *Biochem Biophys Res Commun* 2004; 317: 1045-51.
- 28 Keupp K, Beleggia F, Kayserili H, et al. Mutations in WNT1 cause different forms of bone fragility. *Am J Hum Genet* 2013; 92: 565-74.
- 29 NCBI Gene. LOXL1 lysyl oxidase-like 1 (Homo Sapiens (human)). 2015:.
- 30 Maki JM, Sormunen R, Lippo S, Kaarteenaho-Wiik R, Soininen R, Myllyharju J. Lysyl oxidase is essential for normal development and function of the respiratory system and for the integrity of elastic and collagen fibers in various tissues. *Am J Pathol* 2005; 167: 927-36.
- 31 Maki JM, Rasanen J, Tikkanen H, et al. Inactivation of the lysyl oxidase gene Lox leads to aortic aneurysms, cardiovascular dysfunction, and perinatal death in mice. *Circulation* 2002; 106: 2503-9.
- 32 Kajimura S, Seale P, Kubota K, et al. Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-beta transcriptional complex. *Nature* 2009; 460: 1154-8.
- 33 Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* 2006; 444: 847-53.
- 34 Infante-Rivard C, Weinberg CR, Guiguet M. Xenobiotic-metabolizing genes and small-for-gestational-age births: interaction with maternal smoking. *Epidemiology* 2006; 17: 38-46.
- 35 Pei H, Yao Y, Yang Y, Liao K, Wu JR. Kruppel-like factor KLF9 regulates PPARgamma transactivation at the middle stage of adipogenesis. *Cell Death Differ* 2011; 18: 315-27.
- 36 Khalifa M, Stein J, Grau L, et al. Partial deletion of ANKRD11 results in the KBG phenotype distinct from the 16q24.3 microdeletion syndrome. *Am J Med Genet A* 2013; 161A: 835-40.
- 37 Brandenberger R, Wei H, Zhang S, et al. Transcriptome characterization elucidates signaling networks that control human ES cell growth and differentiation. *Nat Biotechnol* 2004; 22: 707-16.
- 38 Horikoshi M, Yaghoobkar H, Mook-Kanamori DO, et al. New loci associated with birth weight identify genetic links between intrauterine growth and adult height and metabolism. *Nat Genet* 2013; 45: 76-82.
- 39 Weedon MN, Lettre G, Freathy RM, et al. A common variant of HMGA2 is associated with adult and childhood height in the general population. *Nat Genet* 2007; 39: 1245-50.
- 40 van der Valk RJ, Kreiner-Moller E, Kooijman MN, et al. A novel common variant in DCST2 is associated with length in early life and height in adulthood. *Hum Mol Genet* 2015; 24: 1155-68.
- 41 Randhawa R, Cohen P. The role of the insulin-like growth factor system in prenatal growth. *Mol Genet Metab* 2005; 86: 84-90.
- 42 Ishida M, Monk D, Duncan AJ, et al. Maternal inheritance of a promoter variant in the imprinted PHLDA2 gene significantly increases birth weight. *Am J Hum Genet* 2012; 90: 715-9.
- 43 Freathy RM, Mook-Kanamori DO, Sovio U, et al. Variants in ADCY5 and near CCNL1 are associated with fetal growth and birth weight. *Nat Genet* 2010; 42: 430-5.
- 44 Shah S, Bonder MJ, Marioni RE, et al. Improving Phenotypic Prediction by Combining Genetic and Epigenetic Associations. *Am J Hum Genet* 2015; 97: 75-85.
- 45 Horvath S. DNA methylation age of human tissues and cell types. *Genome Biol* 2013; 14: R115.
- 46 Jamshidi Y, Nolte IM, Spector TD, Snieder H. Novel genes for QTc interval. How much heritability is explained, and how much is left to find? *Genome Med* 2010; 2: 35.
- 47 Dehghan A, Dupuis J, Barbalic M, et al. Meta-analysis of genome-wide association studies in >80 000 subjects identifies multiple loci for C-reactive protein levels. *Circulation* 2011; 123: 731-8.
- 48 Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet* 2008; 371: 75-84.
- 49 Nelissen EC, van Montfoort AP, Dumoulin JC, Evers JL. Epigenetics and the placenta. *Hum Reprod Update* 2011; 17: 397-417.



# CHAPTER 4

SUPPLEMENTARY MATERIAL



SUPPLEMENTARY TABLE 1. Cohort descriptives

Cohort	N	Ancestry (%)	Maternal age (years) Mean (SD)	Maternal smoking during pregnancy N (%)		Maternal educational level N (%)				Parity N (%)		
				Yes	No	Level 1	Level 2	Level 3	Level 4	0	≥1	
ALSPAC	633	European	29.9 (4.3)	78 (12.3%)	555 (87.7%)	483 (76.3%)	150 (23.7%)	-	-	-	287 (45.3%)	346 (54.7%)
CHS	199	European	29.5 (5.5)	12 (6.0%)	187 (94.0%)	65 (32.7%)	134 (67.3%)	-	-	-	64 (32.2%)	135 (67.8%)
GECKO	255	European	30.3 (4.2)	129 (50.6%)	126 (49.4%)	175 (68.6%)	80 (31.4%)	-	-	-	90 (35.3%)	165 (64.7%)
Generation R	717	European	31.4 (4.2)	171 (23.8%)	546 (76.2%)	4 (0.6%)	188 (26.2%)	525 (73.2%)	-	-	416 (58.0%)	301 (42.0%)
GOVA	947	European	29.2 (4.0)	139 (14.7%)	808 (85.3%)	119 (12.6%)	357 (37.7%)	471 (49.7%)	-	-	436 (46.0%)	511 (54.0%)
INMA	166	European	30.5 (4.2)	41 (24.7%)	125 (75.3%)	35 (21.1%)	52 (31.3%)	79 (47.6%)	-	-	88 (53.0%)	78 (47.0%)
MoBa1	1025	European	29.9 (4.3)	295 (28.8%)	730 (71.2%)	76 (7.4%)	330 (32.2%)	451 (44.0%)	168 (16.4%)	-	420 (41.0%)	605 (59.0%)
MoBa2	587	European	30.1 (4.4)	144 (24.5%)	443 (75.5%)	46 (7.8%)	193 (32.9%)	236 (40.2%)	112 (19.1%)	-	227 (38.7%)	360 (61.3%)
MoBa3	205	European	29.5 (4.4)	60 (29.3%)	145 (70.7%)	24 (11.7%)	66 (32.2%)	76 (37.1%)	39 (19.0%)	-	100 (48.8%)	105 (51.2%)
NCL	802	European	29.1 (4.9)	257 (32.0%)	545 (68.0%)	99 (12.3%)	703 (87.7%)	-	-	-	325 (40.5%)	477 (59.5%)
NEST	267	European (46.1%) African American (46.4%) Other (7.5%)	28.9 (6.3)	84 (31.5%)	177 (66.3%)	30 (11.2%)	60 (22.5%)	120 (44.9%)	57 (21.3%)	-	85 (31.8%)	181 (67.8%)
PRISM	138	White (41.3%) Black incl. Haitian (38.4%) Hispanic (10.2%) Other (10.1%)	31.1 (5.3)	29 (21.0%)	109 (79.0%)	27 (19.6%)	28 (20.3%)	35 (25.4%)	48 (34.8%)	-	41 (29.7%)	97 (70.3%)

**SUPPLEMENTARY TABLE 2.** Meta-analysis results with Bonferroni corrected genome-wide significant differential DNA methylation in relation to high versus normal birth weight, sorted on p value.

CpG	Effect size	SE	P	Direction	Mapped gene	CHR	Location	BP position	Location
cg20076442	-5.24	0.59	9.30E-19	-----+--		8		72745197	Opensea
cg00049440	-5.32	0.68	4.10E-15	-----+--	KLF9	9	Body	73026643	Shore
cg25124943	-6.32	0.83	2.74E-14	-?------		10		4117248	Opensea
cg25953130	-3.75	0.50	1.14E-13	-----+--	ARID5B	10	Body	63753550	Opensea
cg17714703	6.41	0.91	1.39E-12	+++++++	UHRF1	19	Body	4912221	Shore
cg02863179	-3.49	0.52	1.36E-11	-----	ARID5B	10	Body	63779053	Opensea
cg05387269	5.53	0.82	1.63E-11	++++++		1		1306946	Shelf
cg07144560	-6.52	0.98	2.40E-11	-?------		5		148865756	Opensea
cg11303839	-2.83	0.43	4.66E-11	-----+	CCL26	7	5'UTR	75405967	Opensea
cg13472584	-6.23	0.96	8.21E-11	-----	PRDM2	1	Body	14056972	Opensea
cg14097568	-5.20	0.80	9.49E-11	-----+		1		94792942	Opensea
cg07795325	-5.93	0.93	1.68E-10	-----+--	CAMK2D	4	Body	114557667	Opensea
cg00531137	-4.67	0.74	2.35E-10	-----		16		57643932	Opensea
cg06673684	3.66	0.58	3.15E-10	++++++	MCF2L	13	Body	113646815	Shore
cg03747456	-5.17	0.83	3.62E-10	-----+--	KRT80	12	TSS1500	52586127	Opensea
cg07677157	-3.34	0.54	4.97E-10	--?-----?		12		66050928	Opensea
cg04872675	5.00	0.81	5.97E-10	+++++++		12		108983234	Opensea
cg21121843	-4.05	0.67	1.24E-09	-----	HTT	4	Body	3203982	Opensea
cg03486991	-4.87	0.80	1.34E-09	-?------+--		2		127975738	Shore
cg12036735	-3.96	0.66	1.68E-09	-?------+--		8		20169916	Opensea
cg08404702	3.95	0.66	2.05E-09	++++++	TSC2	16	Body	2133388	Shore
cg13492133	4.42	0.74	2.69E-09	+++++++	SMG7	1	Body	183516266	Opensea
cg27168858	-4.77	0.80	2.82E-09	-----+--	DHCR24	1	Body	55351659	Shore
cg03527802	6.22	1.05	3.39E-09	+++++++	MAZ	16	Body	29819931	Shore
cg02402882	-5.34	0.91	4.72E-09	-----		5		56620429	Opensea
cg09294084	3.86	0.66	5.72E-09	++++++	MCF2L	13	Body	113646732	Shore
cg04521626	-3.99	0.69	9.57E-09	-----+	PLD2	17	Body	4714200	Shelf
cg07807757	-5.70	0.99	9.62E-09	-?------		7		50894570	Opensea
cg13280041	4.84	0.85	1.35E-08	++++++	NHSL1	6	Body	138866865	Opensea
cg12798040	4.61	0.81	1.36E-08	+++++++	XRCC3	14	Body	104171840	Opensea
cg10317026	-10.98	1.94	1.42E-08	-----+	EFNA3	1	Body	155056636	Shore
cg20068209	-3.40	0.61	1.95E-08	-?------+--	TMEM30A	6	Body	75988568	Opensea
cg25144135	-6.05	1.08	2.19E-08	-----+		1		1144012	Shore
cg23847843	3.52	0.63	2.20E-08	+?++++++	ZSCAN23	6	TSS1500	28412459	Opensea
cg12259522	6.73	1.20	2.28E-08	+++++++		13		112310720	Opensea
cg20752878	4.26	0.76	2.34E-08	++++++	ASGR1	17	Body	7080538	Shelf
cg00119811	-7.39	1.33	2.48E-08	+-----	RGNEF	5	Body	73092353	Opensea
cg24173182	-3.16	0.57	2.50E-08	-----	HIC1	17	Body	1961286	Island

**SUPPLEMENTARY TABLE 2.** Meta-analysis results with Bonferroni corrected genome-wide significant differential DNA methylation in relation to high versus normal birth weight, sorted on p value.

CpG	Effect size	SE	P	Direction	Mapped gene	CHR	Location	BP position	Location
cg13586051	-4.62	0.83	2.66E-08	-----+--	DNASE1L3	3	TSS200	58200471	Opensea
cg24559796	-4.39	0.80	3.63E-08	-----+++		7		28223298	Shelf
cg10950251	-3.94	0.72	3.89E-08	-?+-----		1		204466432	Shelf
cg07408552	-6.58	1.20	3.90E-08	+-----	ATP6V0A1	17	5'UTR	40611995	Shore
cg26438325	-4.76	0.87	4.06E-08	-?-----	JAZF1	7	Body	28176718	Opensea
cg14731462	-4.38	0.80	4.93E-08	-----	PTPRE	10	5'UTR	129789429	Opensea
cg27217742	5.19	0.95	5.19E-08	+++++++	RGS12	4	Body	3365280	Island
cg12573002	-7.68	1.41	5.70E-08	-----+	NCR3	6	5'UTR	31560624	Opensea
cg22246785	-4.71	0.87	6.44E-08	-----		12		122021013	Shelf
cg14161821	-3.39	0.63	7.07E-08	-----+--	KDM4C	9	Body	6955559	Opensea
cg27115863	-6.64	1.23	7.24E-08	-----		22		37921640	Opensea
cg24304210	-4.08	0.76	7.46E-08	-----+--	PAK1	11	5'UTR	77118761	Shelf
cg12022621	-3.99	0.74	7.50E-08	-----+	LAX1	1	1stExon	203734505	Opensea
cg13725803	-3.33	0.62	7.60E-08	---+---+	GUCY1B2	13	TSS200	51640297	Opensea
cg05548469	-3.84	0.72	8.10E-08	+-----	TRPC3	4	1stExon	122853464	Shore
cg11724366	-4.35	0.81	9.06E-08	+++-----	FSTL3	19	3'UTR	681815	Shore
cg22457769	-3.33	0.62	9.07E-08	+-----+--		8		144822917	Shore
cg06128598	-5.74	1.08	9.76E-08	-?-?---?--	PYY2	17	TSS1500	26552870	Shore
cg03796381	-4.24	0.80	1.04E-07	-----		15		36650033	Opensea
cg00442282	-7.99	1.50	1.04E-07	-----	RARA	17	5'UTR	38471064	Shore

\*Meta-analysis results of the association between DNA methylation in newborn cord blood and high versus normal birth weight, adjusted for covariates. High birth weight: >4000g and normal birth weight: 2500-4000g. Selected genome-wide significant CpGs after Bonferroni correction. Results sorted by the p-values of associations with birth weight. Column headers: CpG: Cytosine-phosphate-Guanine site; Effect size: regression coefficient; SE: standard error; P: P-value; Direction: Direction of effect across cohorts included in the statistical model: maternal smoking during pregnancy associated with increased (+) or decreased (-) methylation, or missing result (?), in alphabetical order of cohorts; Mapped Gene: UCSC annotated gene; CHR: chromosome; Location in gene: UCSC gene region feature category; BP position: basepair position; Location: CpG islands/shores/shelves/open sea.