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Maternal Preconception Body Mass Index and Offspring Cord Blood DNA Methylation: Exploration of Early Life Origins of Disease

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Abstract

Maternal obesity is associated with a variety of common diseases in the offspring. One possible underlying mechanism could be maternal obesity induced alterations in DNA methylation. However, this hypothesis is yet to be tested. We performed epigenomic mapping of cord blood

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AUTHOR CONTRIBUTIONS

Dr. Xin Liu had the primary responsibility for this manuscript. Drs. Xin Liu, Qi Chen, and Ying Zhou conducted data analysis; Drs. Chunling Zhang and Chunyu Liu provided technical support on the analyses to remove batch and positional effects; Drs. Guoying Wang, Xiumei Hong, Rong Liu, Hongjian Wang, Shanchu Zhang, Yunxian Yu, Karen Mestan, Colleen Pearson, and Peters Otlans all played a role in the acquisition of clinical and experimental data. Dr. Xin Liu drafted the manuscript. Drs. Xiaobin Wang, Karen Mestan, Chunyu Liu, Hui-ju Tsai, and Barry Zuckerman were involved in the interpretation of results and revision of the manuscript. Dr. Xiaobin Wang has been the principal investigator of the Boston Birth Cohort (the parent study), and secured the funding and oversaw the generation of the data for this manuscript. All authors have read and approved the final manuscript.

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among 308 Black mother-infant pairs delivered at term at the Boston Medical Center using the Illumina HumanMethylation27 BeadChip. Linear regression and pathway analyses were conducted to evaluate the associations between DNA methylation levels and prepregnancy maternal BMI (<25, 25–30, ≥30 kg/m^2). The methylation levels of 20 CpG sites were associated with maternal BMI at a significance level of *P*-value <10^{-4} in the overall sample, and boys and girls, separately. One CpG site remained statistically significant after correction for multiple comparisons (FDR corrected *P*-value = 0.04) and was annotated to a potential cancer gene, *ZCCHC10*. Some of the other CpG site annotated genes appear to be critical to the development of cancers and cardiovascular diseases (i.e., *WNT16, C18orf8, ANGPTL2, SAPCD2, ADCY3, PRR16, ERBB2, DOK2, PLAC1*). Significant findings from pathway analysis, such as infectious and inflammatory and lipid metabolism pathways, lends support for the potential impact of maternal BMI on the above stated disorders. This study demonstrates that prepregnancy maternal BMI might lead to alterations in offspring DNA methylation in genes relevant to the development of a range of complex chronic diseases, providing evidence of trans-generational influence on disease susceptibility via epigenetic mechanism.

Keywords

maternal BMI; cord blood; DNA methylation; early life origins of disease

INTRODUCTION

Emerging evidence supports the concept that physiological traits in childhood and adult life can result from both pre- and postnatal risk factors, and that such influence could shape phenotypic plasticity throughout the life course. This idea, known as the developmental origins of health and disease (DOHaD) hypothesis, has been well-recognized in the pathogenesis of common complex diseases [Swanson et al., 2009; Wadhwa et al., 2009]. Epigenetic modifications are the molecular signatures underlying the early life origins of chronic disease via the regulation of gene expression through DNA methylation, histone modifications, chromatin structure remodeling, and actions of noncoding RNAs (ncRNAs) [Golbabapour et al., 2011]. Unlike DNA sequences, the epigenome is both tissue- and cell-specific and time-sensitive, which amplifies the difficulty of epigenetic studies; while the reversible predisposition of the epigenome makes the epigenetic process the focus of etiological and clinical prevention studies. Epigenetic marks are established in utero through initial epigenetic reprogramming during gametogenesis, genome-wide demethylation after fertilization, and de novo genome-wide methylation in the zygote, and then lead to tissue-specific methylation patterns, which can be largely maintained but also can be altered by environmental exposures before and/or after birth [Feinberg, 2008; Hussain, 2012]. As such, the intrauterine period is marked as a highly sensitive and critical window for the formation of epigenetic variations.

The propensity for alterations caused by environmental exposures in utero has triggered numerous studies on the effect of maternal nutrition during pregnancy on epigenetic formation and alterations, but, so far, has been mainly in animals [Milagro et al., 2009; Gong et al., 2010; Widiker et al., 2010; Simmen and Simmen, 2011]. A few studies in

humans have demonstrated that maternal age, race, smoking, and gestational age can contribute to cord blood or placental DNA methylation variations [Adkins et al., 2011a; Adkins et al., 2011b; Schroeder et al., 2011]. The role of maternal prepregnancy body mass index (BMI) in offspring epigenetic variations assessed at birth is yet to be determined.

Maternal obesity is an escalating global problem and has been linked with an increased risk of obesity [Stuebe et al., 2009], autism [Krakowiak et al., 2012], attention-deficit/hyperactivity disorder (ADHD) [Buss et al., 2012], and type 2 diabetes [Dabelea et al., 2008] in the offspring. To date, one study in a U.S. mixed population of over 300 mother-infant pairs demonstrated that obese mothers (BMI ≥ 30kg/m^2) were marginally associated with higher global DNA methylation levels than those with normal weight (BMI = 20–29.9 kg/m^2); however, there was no statistically significant association between prepregnancy BMI and cord blood global DNA methylation of long interspersed nucleotide elements (LINE-1) [Michels et al., 2011]. Whether prepregnancy BMI induces changes in DNA methylation at any specific CpG sites, particularly in the promoter regions on a genome-wide scale, remains to be revealed.

Similar to genome-wide association studies, epigenetic variations have been under-studied in African Americans, who have a higher risk of obesity, high blood pressure, and other cardiovascular diseases compared to Whites.

To address these noted research gaps, this study investigated the effects of prepregnancy BMI on the offspring DNA methylation levels of over 27k CpG sites predominantly located in the promoter regions of ~15,000 genes in Black mother-infant pairs. Not only did we conduct a test for each single CpG site but we also performed pathway analysis of a set of genes to comprehensively understand the contributions of maternal obesity to offspring epigenetic variations at birth.

MATERIALS ANDMETHODS

Study Subjects

This study consisted of 309 Black (African American and Haitian), full-term (≥ 37 gestational weeks) children, who are part of an ongoing epigenomic study of food sensitization conducted in 400 children with mixed ethnicity and a broad range of gestational ages (25.3 to 43.7 gestational weeks, with 82 infants born at less than 37 gestational weeks). The 309 study children are comparable to the 400 children with regards to their other characteristics (i.e., maternal age, education level, marital status, parity, and infant gender) (data not shown). These children were a subset of the Boston Birth Cohort (BBC), who were enrolled after birth at the Boston Medical Center together with their mothers, and then were followed prospectively from birth onward. The population characteristics of the BBC have been published [Wang et al., 2002; Liu et al., 2011; Wang et al., 2012]. We obtained informed consent from the mothers of all study children, and Institutional Review Board approval from the Ann & Robert H. Lurie Children's Hospital of Chicago, the Boston Medical Center and Johns Hopkins University Bloomberg School of Public Health.

Maternal Prepregnancy BMI

Maternal prepregnancy BMI (kg/m^2) was calculated based on self-reported weight and height obtained from the maternal postpartum questionnaire interview. A previous study on a subset of mothers from this birth cohort demonstrated the accuracy of reported BMI, observing a high Pearson correlation coefficient (0.87) between the first trimester BMI obtained from the obstetric clinic and the prenatal BMI obtained from the questionnaire [Kumar et al., 2010]. We grouped 309 subjects into three categories: reference (<25), overweight (25 ≤ BMI < 30), and obese (≥30). Of note, only eight mothers weighed less than 18.5 kg/m^2, and thus were included in the reference group.

Epigenetic Mapping and Quality Control/Quality Assurance

We extracted genomic DNA from whole blood leukocytes and quantified the concentration with SpectraMax M2 (http://www.moleculardevices.com/). Epigenomic mapping was conducted using the Illumina HumanMethylation27BeadChip at the Genomics Core facility, Center for Genetic Medicine, Northwestern University. A total of 14 samples were randomly selected to serve as duplicates and showed high technique consistency with Pearson correlation coefficients >0.99. We dropped one sample due to lower than 98% CpG call rates (i.e., <27,000 probes) and focused on the remaining 308 subjects throughout the study. We sequentially excluded 6,852 probes as follows: 3,011 nonspecific cross-reacting probes identified by Chen et al. [Chen et al., 2011] and our team; 3,674 probes carrying common SNPs (i.e., MAF > 1%); and 167 probes with a detection *P*-value greater than 0.05 for more than 20% of the samples. We then removed potential batch/chip effects with ComBat software, as detailed in previous publications [Zhang et al., 2010; Liu et al., 2011], and further removed potential chip position effects (i.e., 12 locations on the chip) with the same procedure. Association tests for batch/chip and position confirmed that both artifacts were removed completely.

Statistical Analysis

We performed the analyses stratified by infant gender because of the well-known impact of gender on epigenetic patterns [Liu et al., 2010]. Specifically, we conducted quantile normalization for the methylation measures of each probe, and then fit a generalized linear model to examine the associations between quantile normalized DNA methylation levels at each CpG site (dependent variables) and categorized prepregnancy BMI (independent variable) in the overall sample (with the adjustment of gender) and then separately for boys and girls. Categorized maternal age at delivery was included in the regression model given that parental age, particularly maternal age, is associated with DNA methylation levels in the newborn [Adkins et al., 2011b]. We considered an epigenetic association to be significant based on a global test for the two levels of prepregnancy BMI (overweight and obese) after correction for multiple testing using the false discovery rate (FDR) method (PROC MULTTEST) (i.e., FDR-corrected $P < 0.05$). The CpG sites associated with prepregnancy maternal BMI were annotated to the corresponding genes based on an Illumina designed document, and then were searched for functions and diseases in both the Ingenuity Knowledge Base and PubMed.

We also conducted pathway analyses using GSA-SNP software [Nam et al., 2010]. Specifically, each gene was first assigned with the minimum *P*-value of the above association tests for all CpG sites mapped to the gene, and then enrichment analyses were conducted using three canonical pathway (CP) databases enclosed within the Molecular Signatures Database (MSigDB) (http://www.broadinstitute.org/gsea/msigdb/index.jsp): KEGG, BioCarta, and REACTOME. We restricted the analyses to CPs with 10–100 genes to eliminate the bias due to under or over represented genes covered by the applied platform. Gene sets with a Benjamini and Hochberg False Discovery Rate less than 0.05 were reported.

RESULTS

Table I summarizes the epidemiological characteristics of the study subjects ($N = 308$) by prepregnancy maternal BMI status. Mothers who were married or who gave birth to a non-first baby were significantly heavier than those not married or who had their first baby at the time of enrollment, respectively. In addition, mothers who had a higher BMI appeared to have heavier babies.

When looking at the overall sample, only one CpG site (cg01422136) showed significant epigenetic association with maternal BMI after adjustment for maternal age and infant gender (*P*-value for global test of overweight and obese = 1.81×10^{-6} (FDR-corrected *P*-value = 0.04). When boys and girls were examined separately, none of the CpG sites remained statistically significant after correction for multiple testing (FDR-corrected *P*-values >0.05). Table II lists the top hits based on a nominal *P*-value of $<10^{-4}$ either for the global test of two BMI categories or for the test of any BMI category (overweight vs. BMI <25 kg/m^2 or obese vs. BMI <25 kg/m^2). The majority of the genes were identified from the overall sample (i.e., *ZCCHC10, WNT16, ACPL2, C18orf8, ANGPTL2, SAPCD2, ADCY3*) and from boys only (i.e., *PRR16, ERBB2, BTN3A1, GPRC5B, HEYL, POGZ, GSG2, DOK2, CAB39, ANAPC7, MRPL35, ZFP95*) while one was a girl-specific (*PLAC1*) CpG site for which the methylation levels were influenced by maternal BMI. Genes annotated by these CpG sites appear to be critical to the development of cancers, cardiovascular disease, bone mineral density (BMD), endocrine and reproductive system disorders, etc. (Table II). The effect estimates of maternal BMI on these 20 CpG sites are detailed in Supporting Information Table 1. The distributions of the methylation levels (% methylation) of each CpG site listed in Table II are detailed in Supporting Information Figure 1. Compared to mothers with prepregnancy BMI <25 kg/m^2, obese or overweight mothers showed lower DNA methylation levels for the majority of the top hit CpG sites, with a few exceptions (i.e., *ACPL2* (cg00400028), *ADCY3* (cg17644208), *CAB39* (cg06874144)).

No statistically significant pathways were identified after the adjustment for multiple comparisons when examining all subjects as a whole. However, significant maternal prepregnancy BMI associated CPs were identified from KEGG, BioCarta, or REACTOME for boys and girls, separately (Table III). Findings from gender-specific CPs suggested the possibility of differential influences of maternal BMI on the DNA methylation patterns of girls and boys at birth. Specifically, prepregnancy maternal BMI might play a more critical role in infection and inflammation for boys; while not only infectious and inflammatory

response pathways, but also lipid metabolism, cardiovascular diseases, cancers, and endocrine system disorders are indicated for girls.

It should be noted that although all of the study subjects were Black children, to limit the confounder effects from hidden population stratification we included individual ancestry estimates from 141 ancestry-informative markers (AIMs) [Liu et al., 2011] as covariates in the regression model (12 missing information; see footnote of Table I) and found similar results (data not shown) to those listed in Table II. In addition, the findings from the site-level analyses in the overall sample were not dramatically changed when we excluded eight subjects whose mothers had much lower prepregnancy BMI (<18.5 kg/m^2), or excluded 22 subjects exposed to persistent maternal smoking during pregnancy (data not shown). The results also were similar when we further adjusted for offspring birth weight (data not shown).

DISCUSSION

To our knowledge, this is the largest epigenetic study of the effect of maternal prepregnancy BMI on the offspring DNA methylation levels assessed from cord blood in a U.S. Black population. We found that maternal prepregnancy BMI was associated with offspring DNA methylation levels of the CpG sites in genes involved in a broad array of chronic diseases, including cancers, cardiovascular diseases, and inflammation-mediated disorders. Our study suggests that maternal BMI induced alteration in DNA methylation may be one of the mechanisms underlying fetal origins of adult diseases. Moreover, our data suggest that such impact might vary by the gender of the offspring. These findings, if confirmed, should not only provide important insights into the underlying molecular mechanisms regarding how maternal obesity contributes to the development of a variety of common complex diseases in their offspring but also offer strong evidence to inform clinical and public health practice. For example, controlling and preventing maternal obesity would be beneficial for both mothers and their offspring, and epigenetic markers may be used for assessing the effects of intervention.

Obesity in women has been documented to be associated with adverse reproductive outcomes, depression, cardiovascular events, multiple cancers, and all-cause mortality [Kulie et al., 2011; Reynolds et al., 2013]. This study demonstrated that maternal BMI by and large was inversely associated with lower DNA methylation levels of CpG sites mainly at the promoter regions in genes that are relevant to the development of aforementioned diseases. These epigenetic modifications can affect expression of offspring genes starting from birth and could have synergistic effects with offspring postnatal risk factors and obesity/overweight status to increase the risk of disease later on.

The notion that maternal obesity could contribute to the development of cancer in the offspring has emerged from findings showing multiple cancer-related hits (i.e., *WNT16, C18orf8, ANGPTL2, SAPCD2, ADCY3, ERBB2, DOK2, PLAC1*) in tests of single CpG sites (Table II) and findings from pathway-level analysis for a set of genes (Table III). Such inference in general was less likely to be influenced by multiple testing due to various CpG sites per gene or various genes in each pathway, though the conclusion for any single CpG/

gene or pathway might not be definite. For example, one (cg19752722) out of 11 CpG sites in the *ERBB2* gene showed significant association with maternal BMI, and thus could represent a false-positive due to chance alone. However, only one or two CpG sites were tested for other top hits, which could eliminate false positive findings due to the varied numbers of CpG sites being tested per gene. Gene *ZCCHC10* is annotated with the top hit with an FDR corrected *P*-value of 0.04, but has yet to be linked with any disease. Nevertheless, eight proteins that physically interact with ZCCHC10 [Rual et al., 2005; Stelzl et al., 2005] have been linked with several disease categories according to IPA Core Analysis (Supporting Information Figure 2), and five ZCCHC10-interact molecules (i.e., TP53, LUC7L2, EEF1A1, DPPA2, PSME3) have been found to be involved in cancers. Furthermore, the possible influence of maternal BMI on carcinogenesis was likewise evidenced by the pathway-based analyses. Boy-specific pathways emphasized the effects of maternal BMI on several infection and inflammation pathways as well as tumorigenesis and apoptosis pathways. Such evidence also was found in girl-specific pathway analysis. In addition, a single point test in girls identified an X-linked gene, *PLAC1*, which has been demonstrated to be expressed in a variety of human cancers [Fant et al., 2010].

Single point and pathway analyses suggested that prepregnancy BMI may also be associated with the development of cardiovascular, reproductive and endocrine system disorders. For example, *PLAC1* is an essential molecule for placental and fetal development, and its expression in the placenta could have the potential to serve as a predictive biomarker of specific pregnancy complications [Fant et al., 2010]; neuregulin-1 (NRG1)-ERBB2/ERBB4 signaling is involved in cardiomyocyte proliferation and thus controls myocardial growth and regeneration [Wadugu and Kuhn, 2012]. Lipid metabolism pathways observed in girls provide further support that maternal BMI plays an essential role in the development of cardiovascular and endocrine system diseases in the offspring.

Although an imperative role of maternal BMI on cancers and cardiovascular diseases was suggested by both single CpG site and pathway analyses, our interpretation of these findings should still be cautious. First of all, this study used 75% of the samples included in an ongoing epigenomic study of food sensitization, and thus may not be representative of samples from a parental study or from the general population. However, we found no association between food sensitization and prepregnancy BMI, using either categorized variables or continuous measures (data not shown). Second, we could not conduct an in-depth examination in of the possible influence of gestation length on the observed associations due to sample size constraint. However, none of the top genes listed in Table II have been previously shown to be associated with gestational age using the Illumina 27k BeadChips [Schroeder et al., 2011] or the comprehensive high-throughput array-based relative methylation (CHARM) approach [Lee et al., 2012]. Third, this study was limited by the original design, which used chips with a lower coverage of CpG sites for each gene as well as a lower coverage of genes in the genome (~15k genes, <50% known human genes). Fourth, DNA methylation differences across BMI categories for the top hits listed in Table II are small with mean beta values (% methylation) less than 5% (Supporting Information Figure 1), which is similar to previous reports on DNA methylation in cord blood samples [Schroeder et al., 2012; Smith et al., 2012; Koestler et al., 2013]. The biological significance

of the small DNA methylation differences across BMI categories remains to be determined. Fifth, significant findings from pathway analysis suggested that prepregnancy BMI may influence the methylation levels for a set of genes with a moderate effect rather than influence the methylation levels for one or two genes with a major effect. Alternatively, what we found could represent a false positive due to multiple comparisons when using three canonical pathway databases. Finally, possible misclassification of maternal prepregnancy BMI may still exist, even though there is high correlation between maternal prepregnancy BMI based on self-reported weight and height and the first trimester BMI from clinical measurement (Methods). Future studies in a larger sample and using denser chips are needed.

In summary, we found that maternal prepregnancy BMI was associated with offspring DNA methylation levels of the CpG sites in genes involved in a broad array of chronic diseases. Given that maternal BMI is a modifiable factor, our findings, if further confirmed, may have important implications for the primary prevention of chronic diseases. Weight control, particularly among childbearing-aged women, is not only beneficial for expectant mothers but also for their children in the long run. Our study suggests that maternal BMI induced alteration in offspring DNA methylation may be one of the mechanisms underlying the fetal origins of chronic diseases, and underscores the need for prevention of chronic diseases across the life span and generations.

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TABLE I

Epidemiological Characteristics of 308 Black Infants in the Boston Birth Cohort by Maternal Prepregnancy BMI

	BMI < 25 (*N* = 130)	25 ≤ BMI <30 (*N* = 98)	BMI ≥ 30 (*N* = 80)
Variables		**N (%)**	
Maternal age (yrs)			
<20	10 (8)	6 (6)	8 (10)
20–25	30 (23)	15 (15)	16 (20)
25–30	35 (27)	30 (31)	18 (23)
30–35	33 (25)	22 (22)	27 (34)
≥35	22 (17)	25 (26)	11 (14)
Maternal education			
Middle school	30 (23)	28 (29)	27 (34)
High school	43 (33)	38 (39)	23 (29)
> High school	57 (44)	32 (33)	30 (38)
Married[a]	51 (39)	49 (50)	23 (29)
Null parity[a]	63 (49)	59 (61)	54 (68)
Maternal smoking during pregnancy	10 (8)	3 (3)	9 (11)
Infant gender (Male)	71 (55)	47 (48)	37 (46)
		Mean ± SD	
Gestational weeks	39.15 ± 1.21	39.44 ± 1.27	39.47 ± 1.20
Birth weight[b]	3115 ± 471	3262 ± 474	3449 ± 524
African ancestry proportion[a,c]	0.84 ± 0.13	0.85 ± 0.15	0.82 ± 0.12

[a] <0.05

[b] <0.001

[c] Missing in 12 samples.

TABLE II

Top Hits of Differential Methylation Levels for CpG Sites in Cord Blood Associated with Maternal Prepregnancy BMI ($P < 10^{-4}$) in the Boston Birth Cohort

Target ID	Symbol[a]	Beta ± se[b]	P-value[c]	$P_{(df = 2)}$	Gene description	Associated human diseases[a]
cg01422136	ZCCHC10	−0.08 ± 0.13; −0.68 ± 0.14	0.51; 7.80×10^{-7}	1.81×10^{-6}	zinc finger, CCHC domain containing 10	Not found
cg24849648	WNT16	0.14 ± 0.13; −0.48 ± 0.14	0.27; 5.98×10^{-4}	8.38×10^{-5}	wingless-type MMTV integration site family, member 16	Multiple cancers [Manoukian and Woodgett, 2002]; Bone Mineral density [Zheng et al., 2012]; Diabetic Kidney Disease
cg00400028	ACPL2	0.56 ± 0.13; 0.19 ± 0.14	1.69×10^{-5}; 0.17	8.79×10^{-5}	acid phosphatase-like 2	Not found
cg18175410	C18orf8	−0.21 ± 0.13; −0.60 ± 0.14	0.10; 2.47×10^{-5}	1.35×10^{-4}	Chromosome 18 open reading frame 8	Chronic pancreatic; pancreatic cancer
cg11213150	ANGPTL2	−0.33 ± 0.13; −0.55 ± 0.14	0.01; 8.87×10^{-5}	2.81×10^{-4}	Angiopoietin-like 2	Metastatic colorectal cancer
cg15785720	SAPCD2	−0.52 ± 0.13; −0.16 ± 0.14	8.66×10^{-5}; 0.24	4.06×10^{-4}	Suppressor APC domain containing 2	Gastric cancer [Xu et al., 2007]
cg17644208	ADCY3	0.27 ± 0.13; 0.55 ± 0.14	0.04; 9.33×10^{-5}	4.29×10^{-4}	Adenylate cyclase 3	Breast cancer
cg25584626	PRR16	−0.38 ± 0.18; −0.89 ± 0.19	0.03; 7.28×10^{-6}	3.60×10^{-5}	proline rich 16	Coronary Artery Disease
cg19752722	ERBB2	−0.57 ± 0.18; −0.78 ± 0.19	1.63×10^{-3}; 6.90×10^{-5}	7.25×10^{-5}	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2	Multiple cancers; Cardiovascular Disease [Wadugu and Kuhn, 2012]
cg01840268	BTN3A1	−0.60 ± 0.18; −0.78 ± 0.19	1.10×10^{-3}; 9.00×10^{-5}	7.10×10^{-5}	butyrophilin, subfamily 3, member A1	Insulin-dependent Diabetes Mellitus; Huntington's Disease; Rett Syndrome
cg20312475	GPRC5B	−0.32 ± 0.18; −0.85 ± 0.20	0.08; 2.69×10^{-5}	1.41×10^{-4}	G protein-coupled receptor, family C, group 5, member B	Not found
cg25462291	HEYL	−0.49 ± 0.18; −0.78 ± 0.19	6.86×10^{-3}; 9.51×10^{-5}	1.94×10^{-4}	Hairy/enhancer-of-split related with YRPW motif-like	Not found
cg03271651	POGZ	−0.42 ± 0.18; −0.82 ± 0.20	0.02; 5.28×10^{-5}	1.95×10^{-4}	Pogo transposable element with ZNF domain	Not found
cg19585196	GSG2	−0.04 ± 0.18; −0.79 ± 0.20	0.83; 8.78×10^{-5}	2.13×10^{-4}	Germ cell associated 2	Not found
cg03732056	DOK2	−0.32 ± 0.18; −0.81 ± 0.19	0.07; 4.62×10^{-5}	2.29×10^{-4}	Docking protein 2	Lung cancer [Berger et al., 2010]
cg06874144	CAB39	0.75 ± 0.18; 0.24 ± 0.19	4.54×10^{-5}; 0.21	2.32×10^{-4}	Calcium binding protein 39	Huntington's Disease
cg04062907	ANAPC7	−0.36 ± 0.18; −0.80 ± 0.20	0.05; 6.52×10^{-5}	2.86×10^{-4}	Anaphase promoting complex subunit 7	Not found
cg15645605	MRPL35	−0.19 ± 0.18; −0.78 ± 0.19	0.30; 8.64×10^{-5}	4.07×10^{-4}	Mitochondrial ribosomal protein L35	Not found
cg20122491	ZFP95	−0.19 ± 0.18; −0.79 ± 0.20	0.30; 9.29×10^{-5}	4.36×10^{-4}	Zinc finger with KRAB and SCAN domains 5	Not found
cg14674582	PLAC1	−0.12 ± 0.18; −0.80 ± 0.19	0.53; 5.08×10^{-5}	1.20×10^{-4}	placenta-specific 1	Multiple cancers and Preeclampsia [Fant et al., 2010]

[a]Genes were identified from the overall sample (*ZCCHC10, WNT16, ACPL2, C18orf8, ANGPTL2, SAPCD2, ADCY3*), boys (*PRR16, ERBB2, BTN3A1, GPRC5B, HEYL, POGZ, GSG2, DOK2, CAB39, ANAPC7, MRPL35, ZFP95*), and girls (*PLAC1*).

[b] Linear regression coefficient estimate ± se after adjustment of maternal age and infant gender (only for overall sample). The estimates are presented in order for overweight (25 kg/ m^2 ≤BMI <30 kg/m^2) vs. reference group (BMI <25 kg/m^2) and obese (BMI ≥30 kg/m^2) vs. reference group (BMI <25 kg/m2), respectively.

[c] *P*-values are presented in order for overweight (25 kg/m^2 = < BMI <30 kg/m^2) vs. reference group (BMI <25 kg/m^2) and obese (BMI > = 30 kg/m^2) vs. reference group (BMI <25 kg/m^2), respectively.

[d] From Ingenuity Knowledge Base and PubMed search; gene expression or protein levels or genetic polymorphisms are associated with diseases in humans. ~150 references for the gene *ERBB2* according to Ingenuity Knowledge Base.

TABLE III

Pathway Analyses of the Associations between Prepregnancy BMI and Genome-Wide CpG Methylation Assessed from Cord Blood in the Boston Birth Cohort: Stratified by Gender

Set name	Brief description	Gene count	Set size	*p*-value	Corrected *p*-value
Boys					
BIOCARTA_NFKB_PATHWAY	Regulate genes related to apoptosis, viral replication, tumorigenesis and inflammation	20	23	7.71×10^{-5}	0.01
BIOCARTA_DEATH_PATHWAY	Induction of apoptosis through death receptors	29	33	4.25×10^{-4}	0.04
BIOCARTA_TNFR2_PATHWAY	Cytotoxic to tumor cells and regulate MHC and adhesion molecules	16	18	4.55×10^{-4}	0.04
BIOCARTA_RELA_PATHWAY	Acetylation and Deacetylation of RelA in The Nucleus	15	16	7.23×10^{-4}	0.04
Girls					
KEGG_LONG_TERM_DEPRESSION	Decrease In the synaptic strength between parallel fiber and Purkinje cells	58	70	1.69×10^{-5}	2.72×10^{-3}
KEGG_GNRH_SIGNALING_PATHWAY[a]	Regulate Release of the Gonadotropins	77	101	3.06×10^{-5}	2.72×10^{-3}
KEGG_VASCULAR_SMOOTH_MUSCLE_CONTRACTION[a]	Regulate Contractile state of VSMCs	92	115	1.30×10^{-4}	7.00×10^{-3}
KEGG_ALPHA_LINOLENIC_ACID_METABOLISM	Alpha-Linolenic Acid Metabolism	14	19	3.20×10^{-4}	0.01
KEGG_GAP_JUNCTION[a]	Intercellular Adhesions	65	90	4.44×10^{-4}	0.01
KEGG_LINOLEIC_ACID_METABOLISM	Linoleic acid metabolism	19	29	7.38×10^{-4}	0.02
KEGG_SMALL_CELL_LUNG_CANCER	Small cell lung cancer	78	84	1.11×10^{-3}	0.03
KEGG_ARACHIDONIC_ACID_METABOLISM	Arachidonic acid metabolism	41	58	1.60×10^{-3}	0.03
REACTOME_PLATELET_SENSITIZATION_BY_LDL	Involved in Platelet Sensitization by LDL (Platelet Homeostasis)	12	16	7.31×10^{-6}	3.82×10^{-3}
REACTOME_REGULATION_OF_IFNG_SIGNALING	IFNG signaling Regulation	13	14	7.43×10^{-5}	0.02
REACTOME_INTEGRATION_OF_ENERGY_METABOLISM[a]	Integration of energy metabolism	96	120	1.91×10^{-4}	0.03
REACTOME_BETA_DEFENSINS	Beta defensins (antimicrobial peptides)	10	42	2.59×10^{-4}	0.03

Note. Output from GSA-SNP (http://gsa.muldas.org/). Gene Count: the number of genes found from the input (~15k genes covered by Illumina 27K BeadChip); Set Size: the size of the gene sets from the known databases (KEGG, BioCarta, REACTOME); Correct *P*-value: Benjamini and Hochberg False Discovery Rate.

[a]Canonical pathways include the *ADCY3* gene, which was identified from a single test for CpG site (Table II).