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Exposure to Maternal Diabetes in Utero and DNA Methylation Patterns in the Offspring

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Abstract

Perturbations in early life environments, including intrauterine exposure to maternal gestational diabetes (GDM), are hypothesized to lead to metabolic imprinting resulting in increased risk of cardiometabolic outcomes later in life. We aimed to 1) identify candidate genes and biological pathways associated with differentially methylated regions (DMRs) in relation to exposure to GDM *in utero* and, 2) using mediation analysis, more definitively investigate the potential for mediation of the effect of exposure to maternal diabetes *in utero* on cardiometabolic traits in childhood risk through our identified DMRs. Genome-wide methylation analysis of peripheral blood mononuclear cell's DNA was conducted in 21 healthy children, ages 8-12 years. P-values from multiple linear regression analyses for >27,000 CpG sites were ranked to identify DMRs between the exposure groups. Among the top 10 ranked DMRs, we identified several genes, including NPR1, PANK1, SCAND1, and GJA4, which are known to be associated with cardiometabolic traits. Gene enrichment analysis of the top 84 genes, each with p<=0.005, identified the ubiquitin proteasome system (UPS) as the most enriched biological pathway (p = 0.07). The UPS pathway reflects biological processes known to be associated with endothelial function, inflammation, lipid metabolism, insulin resistance and β-cell apoptosis, whose derangements are central to the pathogenesis of cardiometabolic diseases. Increased methylation of *PYGO1* and *CLN8* had the greatest relative mediation effect (RME = 87%, p=0.005 and RME=50%, p=0.01) on the impact of exposure to maternal diabetes *in utero* on VCAM-1 levels in the offspring. Multiple candidate genes and the UPS were identified for future study as possible links between exposure to maternal gestational diabetes *in utero* and adverse cardiometabolic traits in the offspring. In particular, increased methylation of *PYGO1* and *CLN8* may be biological links between intrauterine exposure to maternal diabetes and significantly increased VCAM-1 levels in the offspring.

Keywords

DNA methylation; Epigenetics; Gestational diabetes; Cardiometabolic; Intrauterine

Introduction

The 'developmental origins of adult health and disease' hypothesis posits that perturbations in early life environments lead to metabolic imprinting resulting in increased risk of

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cardiometabolic outcomes later in life [1]. Several epidemiological studies and animal models have provided substantial evidence that intrauterine exposure to maternal gestational diabetes (GDM) has lifelong effects on the offspring, including an increased risk of obesity, type 2 diabetes (T2D), and cardiovascular disease [2,3]. Exposure to maternal diabetes *in utero* results in increased metabolic substrate delivery to the fetus (i.e., fetal overnutrition). Adverse nutritional exposures *in utero* are hypothesized to influence the long-term patterns of gene expression levels [4]. However, the mechanisms responsible for these long-term effects remain poorly understood.

Epigenetic modifications, such as DNA methylation, can alter gene expression or cellular phenotypes without changes in the underlying DNA sequence. The induction of persistent epigenetic change by prenatal environmental conditions may be a mechanistic link between early development and health and disease in later life in humans. DNA methylation involves the covalent addition of a methyl group to the cytosine base within the context of CpG dinucleotides, which are frequently clustered into CpG islands within regulatory sites of gene promoter regions and functions as a regulator of gene expression [5]. Tightly controlled DNA methylation is essential in early fetal development [6]. Although DNA methylation patterns are responsive to the environment throughout life, the epigenome is particularly susceptible to aberrant methylation during gestation and neonatal development because the DNA synthetic rate is high and the DNA methylation patterning required for normal tissue development is established during this period [7].

Key first steps in investigating the potential mediation of exposure to GDM *in utero* and later life propensity to adverse cardiometabolic traits by abherrant DNA methylation are 1) determine if DNA methylation patterns differ among offspring exposed and not exposed to maternal GDM; 2) determine if differing methylation patterns correspond to genes and biological pathways that have been linked to adverse cardiometabolic risk factors; and 3) investigate if identified DMRs mediate the effect of intrauterine exposure to maternal diabetes on adverse cardiometabolic traits in the offspring. In a pilot study among a retrospective cohort of children exposed and not exposed to maternal GDM *in utero*, we aimed to identify candidate genes and biological pathways associated with differentially methylated regions (DMRs) that may be linked to adverse cardiometabolic risk factors in the child offspring. Additionally, using mediation analysis, our study attempted to more definitively investigate the potential for mediation of the effect of exposure to maternal diabetes *in utero* on cardiometabolic traits in childhood risk through our identified DMRs.

Materials and Methods

Study population

This analysis included a sample of 21 healthy non-Hispanic white children who were participants in the Exploring Perinatal Outcomes among Children (EPOCH) study. EPOCH is a retrospective cohort of children exposed and not exposed to maternal diabetes *in utero*, who were offspring of singleton pregnancies, aged 8-12 years in 2006 to 2009, and whose biological mothers were members of the Kaiser Permanente of Colorado health plan [8]. This study was reviewed and approved by the Colorado Multiple Institutional Review Board and the Kaiser Permanente institutional review board. Written informed consent from the mothers and assent from the children were obtained. For the current study, 11 non-Hispanic white offspring (6 females and 5 males) who had been exposed to maternal gestational diabetes during intrauterine life were randomly selected from a total of 67 exposed offspring within the larger EPOCH cohort. Similarly, 11 offspring (6 females and 5 males) were randomly selected from a total of 267 unexposed offspring within the larger cohort. One of the 22 DNA samples was excluded from the methylation analyses because of poor quality DNA.

Exposure definition

Physician-diagnosed maternal diabetes status was ascertained from the health plan database. Routine screening for maternal gestational diabetes in the non-diabetic pregnancies was performed. At 24–28 weeks, all pregnant women were offered screening for gestational diabetes with a 1 h, 50 g oral glucose tolerance test (OGTT). Women with a value ≥7.7 mmol/l were asked to undergo a 3 h, 100 g diagnostic OGTT. Gestational diabetes was diagnosed when two or more glucose values during the diagnostic OGTT met or exceeded the criteria for a positive test [9]. Exposure to diabetes *in utero* was defined as maternal diabetes diagnosed during the index pregnancy.

Quantification of DNA methylation

The genome-wide methylation analysis was conducted in 11 exposed children and 10 unexposed children. Blood samples were drawn after an overnight fast for measurement of DNA methylation in DNA obtained from peripheral blood mononuclear cells. Bisulphite conversion of genomic DNA was performed using the EZ DNA Methylation™ Kit (©Zymo Research Corp., Irvine, CA, USA) according to the manufacturer's instructions. The DNA methylation profiling was conducted in the bisulphite converted DNA using the Illumina Infinium HumanMethylation27 BeadChip (San Diego, CA, USA). This platform investigates 27,578 individual cytosines at CpG loci throughout the genome with focus on the promoter regions of 14,495 genes. Exposed and unexposed case samples were randomly arranged on each chip in an approximate 50:50 proportion. Internal quality controls were included. Each methylation data point was represented by the ratio of methylated to methylated plus unmethylated fluorescent intensity signals, represented as β values. The β values range from 0 (unmethylated) to 1 (fully methylated) on a continuous scale.

Covariates

Self-reported Tanner staging was used to categorize pubertal development with a diagrammatic representation of staging [10] using scales ranging from 1 (prepubertal) to 5 (adult). The offspring were categorized as Tanner <2 (prepubertal) and ≥2 (pubertal). Caloric intake was ascertained using a modified version of the Block Kid's Food Questionnaire [11], a semi-quantitative usual intake food frequency questionnaire developed specifically for youth aged 8 years and older. Physical activity was obtained by self-report using questions based on the Youth Risk Behavior Surveillance System [12] and was categorized as the average number of 30-minute blocks of moderate-to-vigorous activity per day.

Cardiometabolic risk factor measurements

Childhood and maternal height was measured to the nearest 0.1 cm by stadiometer. Weight was measured to the nearest 0.1kg using an electronic scale. Age- and sex-specific BMI z scores were calculated for the children [13]. Waist circumference, measured to the nearest 0.1 cm at the end of normal expiration just above the right iliac crest at the mid-axillary line, was measured twice and averaged. Two sitting blood pressure measurements were averaged. Blood samples were drawn in the offspring after an overnight fast for measurement of lipids (measured using the Olympus AU400 advanced chemistry analyser system), adiponectin and leptin (measured with enzyme-linked immunosorbent assay kits), and soluble cell adhesion molecules, including vascular adhesion molecule 1 (VCAM-1) and E-selectin (measured by a Luminex multiplex assay kit method, Austin, TX, USA). Laboratory analyses were performed at the University of Colorado Hospital laboratory and the Children's Hospital laboratory (Aurora, CO, USA). LDL concentration was determined by the Friedewald equation [14].

Statistical analyses

Analyses focused on identifying genes corresponding to CpG sites that were differentially methylated between the exposed and unexposed groups of children. The β value from each CpG site was logit-transformed prior to testing for association with maternal diabetes exposure status in offspring. Each logit-transformed β value was regressed on offspring exposure status using multiple linear regression. To correct for the varying number of CpG sites tested within individual genes, the minimum p-value of all tested CpG sites for a gene was evaluated for statistical significance. Using first-order statistics distributional theory, the minimum p-value was assumed to follow a β distribution and a p-value was determined for each gene based on the minimum CpG p-value [15]. The p-values derived from the first-order statistical tests were ranked to identify DMRs among genes between the exposed and unexposed groups, using a pragmatic cutoff value of $p \leq 0.005$.

To increase the precision of the estimates we adjusted for age, sex, pubertal stage, physical activity, and caloric intake of the offspring, which are non-confounding predictor covariates that have been associated with DNA methylation patterns [16-19].

Pathway enrichment analysis

Based on the results from the multiple regression analyses a list of genes corresponding to differentially methylated CpG sites with p-values ≤ 0.005 was generated and tested for enrichment of known biological pathways. The enrichment analysis was done using the Database for Annotation, Visualization and Integrated Discovery (DAVID), a web-accessible gene database that identifies the most overrepresented biological pathways associated with a large set of genes [20,21]. The functional annotation clustering tool was used, which clusters similar annotations based on the co-occurrence of particular gene sets. Functional annotation analysis compared the differentially methylated genes to the background population of genes from the Illumina chip to identify biological pathways most relevant to our gene list. Default parameters in DAVID were used including a modified Fisher exact p-value = 0.1 as the maximum probability [22].

Mediation analysis

To investigate whether our identified DMRs mediated the relationship between exposure to maternal diabetes *in utero* and cardiometabolic traits, we conducted a mediation analysis using the framework proposed by Baron and Kenny [23]. Assessing mediation in this manner involved fitting both an outcome model and a mediator model. The mediator model was a multiple regression model that modeled a cardiometabolic trait as the outcome, included exposure status to maternal diabetes *in utero* as the exposure of interest, and included DMRs as mediators. Each of the top 10 ranked DMRs was assessed for evidence of mediation using a series of multivariate regression models. This approach is based on the principle that controlling for intermediates in the causal pathway between a risk factor and an outcome will reduce the observed effect of the more distal determinant. Evidence of mediation was formally assessed using the relative mediation effect [24], which represents the proportion of the association between exposure to maternal diabetes *in utero* and each cardiometabolic trait that was accounted for by each potentially mediating DMR.

Results

Characteristics of the offspring are described in Table 1, which shows similar age, sex, pubertal stage, calorie intake, and physical activity among the exposed and unexposed children. Table 1 also shows significantly higher BMI z scores and waist circumference as well as higher levels of VCAM-1 and leptin among the exposed offspring compared to the unexposed children. Triglyceride levels and LDL-cholesterol levels were moderately higher

in the exposed children compared to the unexposed. Table 2 shows characteristics of the mothers at the study visit. Mothers who had GDM during the index pregnancy had significantly higher body mass index and waist circumference compared to the non-diabetic mothers.

Using a threshold of $P \leq 0.005$ we identified 84 DMRs corresponding to 84 genes, although no single DMR reached a multiple testing correction threshold of false detection rate (FDR) < 0.05. Table 3 shows the top 84 genes ranked by statistical significance. Of the top 10 genes, 9 showed increased methylation in exposed offspring compared to unexposed children.

The most enriched biological pathway category identified from the list of 84 genes was the ubiquitin proteasome system (UPS) pathway (modified Fisher exact p-value = 0.07).

BMI z scores, waist circumference, VCAM-1 levels, and leptin levels were the cardiometabolic traits that were significantly associated with intrauterine exposure to maternal diabetes in this study so these traits were carried forward for mediation analysis. We also selected the top 10 ranked DMRs as mediators for the analysis. Because we tested 10 DMRs as mediators for each of the cardiometabolic traits we used the Bonferroni correction to adjust the alpha level for multiple comparisons, setting the criteria at $p < 0.005$. None of the DMRs were significantly associated with BMI z scores, waist circumference or leptin levels but *CLN8* (p=0.0046) and *PYGO1* (p=0.009) methylation values were significantly associated with VCAM-1 levels. Table 4 shows significant changes in the parameter estimate for the effect of exposure to maternal diabetes on VCAM-1 levels when DNA methylation values associated with *PYGO1* and *CLN8* were included (separately) as a covariates in the regression models. *PYGO1* had the greatest relative mediation effect (RME = 87%, p = 0.005) on the impact of exposure to maternal diabetes *in utero* on VCAM-1 levels in the offspring. *CLN8* also had a significant but lesser relative mediation effect (RME=50%, p=0.01).

Discussion

Using samples from a contemporary cohort study of children, we identified variations in DNA methylation patterns that are associated with intrauterine exposure to maternal GDM. Of the top 10 DMRs, ranked by statistical significance, we identified several genes that are known to be associated with a variety of cardiometabolic traits. Variants in the NPR1 gene have been associated with higher systolic blood pressure in young adults and with hypertensive disease in animal models, suggesting that abnormalities in expression could play a key role in the pathogenesis of hypertension [25,26]. PANK1 has been associated with fasting insulin levels in a genome-wide association study [27]. Single nucleotide polymorphisms in the SCAND1 gene have been associated with plasma HDL cholesterol levels [28] and SCAND1 has been shown to interact with transcription factors that regulate genes involved in lipid metabolism, suggesting it may be an important co-regulator of such genes [29]. Variations in the GJA4 gene have been associated with myocardial infarction [30] and with carotid intima-medial thickness [31], a surrogate marker of subclinical atherosclerosis.

The most enriched biological pathway identified in our gene set, the UPS, is known to play key roles in processes that are closely related to the initiation and progression of T2D and atherogenesis and are directly associated with risk factors for cardiometabolic diseases. The UPS plays a central role in the intracellular degradation of several proteins that fulfill important functions in the regulation of biological processes including vascular inflammation, oxidative stress, cell proliferation, vascular cell apoptosis, cholesterol

metabolism, and endothelial function [32,33]. Another important role of the UPS includes regulating cell signaling during development of the vascular system, one of the earliest and most pivotal events that occurs during embryogenesis [34]. The UPS also appears to be involved in regulating lipid synthesis in adipocytes and lipid production by the liver, which could influence the development of obesity [35]. Insulin receptor substrate proteins can be ubiquitylated and degraded by the UPS to induce insulin resistance [36,37]. Further, a defect in insulin secretion can occur due to UPS-mediated degradation of insulin substrate receptor-2 in the β-cells of the pancreas [35,38].

The mediation analyses provide evidence that the association between exposure to maternal diabetes *in utero* and increased VCAM-1 levels in the offspring may be mediated via differential methylation of the PYGO1 and CLN8 genes.

There is clear evidence that the early pathogenesis of cardiometabolic diseases is apparent in childhood and that risk factors, which are predictive of future cardiometabolic risk in adulthood, are identifiable in childhood [39]. Endothelial dysfunction within the vasculature is considered to be an early and crucial event in the pathogenesis of atherosclerosis [40]. Endothelial dysfunction is marked by the up-regulation of cellular adhesion molecules, such as VCAM-1, intercellular adhesion molecule 1 (ICAM-1), and selectins [41]. Notably, we report significantly higher levels of VCAM-1 in the exposed group of children in this study and we recently reported substantially increased levels of the circulating endothelial cell adhesion molecules VCAM-1 and E-selectin in child offspring exposed to maternal diabetes in the larger EPOCH study cohort [42].

Several limitations should be considered when interpreting our findings. No single CpG site reached a multiple testing correction threshold of FDR< 0.05, which may be due to the modest size of our sample. Nevertheless, our use of a pragmatic threshold for ranked p-values identified methylation signatures relating to pathogenic processes leading to cardiometabolic dysfunction, which will need to be validated by subsequent analyses. Further, we were not able to obtain measurements of the severity or duration of hyperglycemia in the mothers during the pregnancy; however, we were able to ascertain physician-diagnosed diabetes status during pregnancy from routine screening and exclude mother-child pairs with previously diagnosed type 1 diabetes in the mothers. Although DNA methylation levels are known to vary among tissue and cell types, it has been shown that the nutritional effects of methylation in one tissue are highly correlated with that in other tissues [4]. Our quantification of DNA methylation in peripheral blood mononuclear cells was informative to determine whether any genome-wide methylation signatures could be detected in this easily accessible tissue. Finally, previous reports suggest that DNA methylation is divergent between race/ethnic populations at specific loci [43], thus, results from our sample of non-Hispanic white children may not be generalizable to other populations.

The strengths of this study include the accurate assessment of intrauterine exposure to maternal diabetes and the use of high-throughput investigation of DNA methylation across 27,000 individual CpG sites throughout the genome. Further, we were able to conduct a mediation analysis to relate the DNA methylation patterns to adverse cardiometabolic traits in this cohort of children. Although these results require confirmation in larger cohorts, multiple candidate genes and the UPS were identified for future study as possible links between exposure to maternal gestational diabetes *in utero* and adverse cardiometabolic traits in the offspring. In particular, increased methylation of the PYGO1 and CLN8 genes may be biological links between intrauterine exposure to maternal diabetes and significantly increased VCAM-1 levels in the offspring.

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Table 1

Characteristics of the offspring

Characteristics	Exposure to Maternal Diabetes (n=11)	No Exposure (n=10)	P-value
Age, years (SD)	9.9 (1.4)	10.8 (0.6)	.07
Female, n (%)	6 (55%)	6 (60%)	.80
Tanner stage <2 (prepubertal) ≥2 (pubertal)	7 (64%) 4 (36%)	4 (40%) 6 (60%)	.28
Kilocalorie intake/day (SD)	1826 (508)	1829 (500)	.99
Physical activity * (SD)	2.3 (1.4)	1.8 (1.0)	.39
Systolic blood pressure (mmHg)	103	97	.18
Diastolic blood pressure (mmHg)	70	66	.23
BMI z score	0.8 (1.2)	−0.3 (1.0)	.03
Waist circumference (cm)	68.9 (12.1)	59.7 (5.8)	.04
Triglyceride [a]	99	74	.09
HDL-cholesterol	53	56	.50
LDL-cholesterol	113 (30)	89 (32)	.10
E-selectin (ng/ml)[a]	1.3	1.0	.32
VCAM-1 (ng/ml)	45.8	32.4	.02
Adiponectin (μg/ml)[a]	11.3	12.5	.71
Leptin (ng/ml)[a]	8.2	3.4	.04

* Number of 30-minute blocks of moderate-to-vigorous physical activity per day

[a] Geometric mean reported

Table 2

Maternal characteristics at clinic visit

Characteristics	Maternal Diabetes (n=11)	No Diabetes (n=10)	P-value
Age, years	32.7 (4.4)	32.7 (4.1)	.99
Systolic blood pressure, mmHg	114 (11)	111 (8)	.53
Diastolic blood pressure, mmHg	77 (9)	80 (7)	.50
Body mass index, kg/m^2	30.8 (5.7)	25.3 (3.8)	.02
Waist circumference, cm	99.5 (14.3)	79.8 (7.5)	.001

Data are means (SD) and p-values are from t-tests.

Table 3

Top 84 differentially methylated genes (p≤ 0.005) ranked by statistical significance

Symbol	Chromosome	Mean beta value in exposed/unexposed offspring (%)*	Average % increase in methylation among exposed relative to unexposed offspring*	P-value
NPR1	1	9.3/5.8	59.2	9.1×10^{-5}
ZNF330	4	14.6/10.8	34.9	1.5×10^{-4}
PANK1	10	19.2/12.7	51.0	2.5×10^{-4}
SCAND1	20	6.1/4.0	52.3	4.4×10^{-4}
GJA4	1	7.7/5.8	32.6	5.2×10^{-4}
UBE2D1	10	7.4/4.8	52.6	6.0×10^{-4}
PYGO1	15	5.5/3.8	44.8	6.5×10^{-4}
IL12RB2	1	6.9/5.5	25.3	6.6×10^{-4}
CLN8	8	17.6/10.2	72.4	9.0×10^{-4}
ZDHHC12	9	8.3/15.1	−45.1	9.4×10^{-4}
STX10	19	10.7/7.5	42.0	9.8×10^{-4}
UBADC1	9	10.3/7.1	45.4	0.0010
DCUN1D5	11	3.5/2.5	40.4	0.0011
FAM14B	14	14.4/9.5	52.1	0.0012
TMCO1	1	7.3/4.6	59.8	0.0012
ZDHHC14	6	4.0/3.3	23.3	0.0013
SHMT2	12	5.8/4.0	47.5	0.0013
BFSP1	20	11.1/8.4	33.0	0.0014
CMTM6	3	4.1/3.2	26.5	0.0014
ZNF345	19	24.6/18.6	32.6	0.0015
SPATC1	8	16.5/11.8	40.1	0.0015
GOLPH2	9	12.4/8.5	46.0	0.0016
OAZ2	15	21.5/13.3	62.0	0.0017
HSPBAP1	3	9.3/6.8	37.7	0.0017
TMEM34	4	7.9/5.8	36.4	0.0018
LRPPRC	2	13.0/9.3	39.0	0.0019
NACA	12	5.7/4.0	43.2	0.0021
USP52	12	7.8/5.1	52.2	0.0021
ATP6V1C2	2	5.1/3.3	54.9	0.0022
LUC7L	16	6.0/4.8	26.8	0.0022
SND1	7	7.0/4.5	53.9	0.0023
SYNC1	1	23.6/11.4	106.6	0.0024
DHDDS	1	7.5/5.1	46.6	0.0024
HSPA14	10	5.3/3.5	52.5	0.0026
DNAH3	16	11.4/7.3	57.0	0.0026

Symbol	Chromosome	Mean beta value in exposed/unexposed offspring (%)*	Average % increase in methylation among exposed relative to unexposed offspring*	P-value
TNNT1	19	9.5/21.5	−55.9	0.0026
TSFM	12	8.9/5.7	57.8	0.0026
TOMM34	20	6.3/4.3	46.5	0.0027
TMEM74	8	6.2/4.2	47.3	0.0028
SLC45A3	1	5.5/4.0	38.7	0.0028
LOC57146	16	7.1/5.2	36.0	0.0029
OPA3	19	5.0/3.6	37.7	0.0029
GIMAP2	7	17.0/10.5	62.9	0.0029
WDR69	2	5.2/3.7	38.8	0.0031
MLR2	10	10.6/8.0	32.5	0.0032
LSM14A	19	3.5/2.7	27.7	0.0032
C1orf57	1	8.1/6.0	35.3	0.0033
ZNF426	19	9.9/6.4	54.5	0.0033
CYB561	17	5.5/4.1	32.3	0.0033
PEX14	1	10.3/6.3	64.4	0.0035
NPY1R	4	11.1/8.3	34.2	0.0035
SLC35A3	1	15.3/10.8	41.3	0.0035
PARK7	1	17.4/11.4	52.5	0.0036
UBE2V2	8	8.5/6.3	36.3	0.0037
PSEN1	14	5.1/3.8	35.4	0.0037
LAT	16	4.9/4.2	18.5	0.0038
NCOA6	20	11.7/8.8	32.3	0.0038
ANKS1A	6	5.4/4.1	30.8	0.0038
C2orf28	2	5.9/4.4	36.2	0.0039
UBXD6	8	3.8/2.9	31.2	0.0040
AFF1	4	5.5/4.6	21.6	0.0041
INPP5A	10	19.1/13.4	42.6	0.0041
TES	7	4.3/3.2	37.2	0.0041
CTLA4	2	33.2/39.7	−16.4	0.0042
SLC35B3	6	14.6/9.6	52.4	0.0042
UBE2B	5	8.1/5.3	52.0	0.0042
PPRC1	10	12.9/10.5	23.5	0.0043
PSCD3	7	6.4/4.9	30.8	0.0043
KCTD3	1	16.8/11.1	51.1	0.0044
MTUS1	8	8.4/6.1	37.5	0.0045
IGSF3	1	7.1/5.9	19.2	0.0046
ZNF211	19	6.9/5.5	25.9	0.0046
TMEM128	4	27.7/21.6	28.4	0.0047

Symbol	Chromosome	Mean beta value in exposed/unexposed offspring (%)*	Average % increase in methylation among exposed relative to unexposed offspring*	P-value
ALDH6A1	14	4.9/4.0	23.2	0.0048
RARRES3	11	10.5/7.6	39.2	0.0048
KIAA0776	6	20.2/14.4	40.3	0.0049
PFKFB2	1	9.8/6.6	49.0	0.0049
HPS3	3	20.6/14.0	46.9	0.0050
GCDH	19	4.6/3.4	35.9	0.0051
XPC	3	5.8/4.0	44.7	0.0053
FRAP1	1	12.9/9.8	31.6	0.0053
SCP2	1	13.1/19.3	−32.1	0.0055
MGC34646	8	14.9/9.8	51.4	0.0055
EIF2AK2	2	12.1/8.0	52.1	0.0055

*Adjusted for age, sex, Tanner stage, daily kilocalorie intake, and physical activity. Mean values were back-transformed to the original units.

Table 4

Significant mediation effects of differentially methylated regions for the association between exposure to maternal diabetes in utero and VCAM-1 levels in the offspring

Model	Regression coefficient	Relative mediation effect (%)	P value
Baseline model (exposure status to maternal diabetes)	13.4	--	--
Baseline plus *PYGO1* methylation	1.7	87%	0.005
Baseline plus *CLN8* methylation	6.7	50%	0.01