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Placental maternally expressed gene 3 differentially methylated region methylation profile is associated with maternal glucose concentration and newborn birthweight

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ABSTRACT

Aims/Introduction: Emerging evidence shows that epigenetic modifications occurring during fetal development in response to intrauterine exposures could be one of the mechanisms involved in the early determinants of adult metabolic disorders. This study aimed to investigate whether the placental maternally expressed gene 3 (*MEG3*) deoxyribonucleic acid (DNA) methylation profile is associated with maternal gestational diabetes mellitus status and newborn birthweight.

Materials and Methods: Samples for measurement were collected from 23 women with gestational diabetes mellitus and 23 healthy controls. *MEG3* gene expression and DNA methylation levels were assessed using quantitative real-time polymerase chain reaction and MethylTargetTM, respectively. Pearson correlation analyses were used to examine associations between placental DNA methylation levels and clinical variables of interest. The associated results were adjusted by multivariate linear regression for maternal age, body mass index, height, gestational age and newborn sex as confounders.

Results: We found that the DNA methylation levels in the *MEG3* differentially methylated region were significantly different between the gestational diabetes mellitus and control groups on the maternal side of the placenta (40.64 ± 2.15 vs 38.33 ± 2.92; $P = 0.004$). Furthermore, the mean *MEG3* DNA methylation levels were correlated positively with maternal fasting glucose concentrations ($R = 0.603$, $P < 0.001$) and newborn birthweight ($R = 0.568$, $P < 0.001$).

Conclusions: The placental DNA methylation status in the *MEG3* differentially methylated region was correlated with maternal glucose concentrations and newborn birthweight. These epigenetic adaptations might contribute to late-onset obesity, underlining the adverse intrauterine environment.

INTRODUCTION

Gestational diabetes mellitus (GDM) is a common metabolic disorder, defined as any degree of glucose intolerance with onset or first recognition during pregnancy[1]. The prevalence of GDM is estimated to range from 6.5 to 25%[2,3]. Accumulating studies have shown that GDM could cause short- and long-term complications in both mothers and offspring. Maternal adverse outcomes associated with GDM include pre-eclampsia, postpartum hemorrhage and susceptibility to future metabolic diseases[4,5]. Fetuses exposed to the maternal hyperglycemic environment are at increased risk of stillbirth, premature birth, macrosomia, shoulder dystocia and neonatal respiratory distress syndrome[1,6]. In addition, the offspring of women with GDM are at elevated risk of developing diabetes, cardiovascular disease, overweight and obesity, with potential transgenerational effects[7].

The underlying mechanisms involved in developmental programming are still obscure. One potential molecular

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mechanism that has been proposed to explain the long-term effect of exposure to a deleterious fetal environment is epigenetics. Epigenetics refers to regulating gene expression without altering the nucleotide sequence. Deoxyribonucleic acid (DNA) methylation is the most stable and widely studied epigenetic mechanism, which occurs as the addition of a methyl group to the 5-carbon position of cytosine residues within CpG islands (genomic regions rich in CpG dinucleotides). Abnormal DNA methylation mediated by DNA methyltransferase enzymes might result in inappropriate gene silencing, thus regulating gene expression and subsequent cellular functions.

The placenta is critical for regulating fetal development, producing hormones and responding to environmental signals. Adverse conditions *in utero* might alter placental gene regulation and subsequent placental physiology, consequently, fetal development. A hyperglycemic environment during pregnancy (GDM and pregestational diabetes) disturbs endothelial cell cycle pathways[8], changes inflammatory patterns[9], induces decidual vasculopathy[10] and impacts on vascular permeability of the placenta[11]. Epigenetic adaptations in the placenta makes it specifically susceptible to the environment[12]. Epigenetic modifications in the placenta, mainly carried out by genomic imprinting and DNA methylation, contribute to these short- and long-term outcomes. Previous studies reported dysregulation in placental lipoprotein lipase and *IGF2/H19* DNA methylation exposed to the intrauterine hyperglycemic environment[13,14].

Maternally expressed gene 3 (*MEG3*) is an imprinted gene located on human chromosome 14q32.3 within the *DLK1-MEG3* locus[15]. *MEG3* encodes a long non-coding ribonucleic acid (RNA), and has been reported to participate in various types of human diseases[16–19]. The expression of *MEG3* is regulated by two differentially methylated regions (DMRs) – the intergenic differentially methylated region (IG-DMR) and the *MEG3*-DMR[20,21]. Previous studies have shown that epigenetic modification of the *DLK1-MEG3* domain is altered in type 1 and type 2 diabetes[19,22]. Hypermethylation of *MEG3*-DMR in mice with diabetes results in decreased *MEG3* messenger RNA (mRNA) levels[23]. A genome imprinting study showed that the Dlk1-Dio3 locus is responsible for fetal growth in mice[24]. Hence, the *MEG3* DNA methylation profile appears to have a potential role in GDM-related developmental programming. We supposed that maternal hyperglycemia could alter *MEG3* DNA methylation status, possibly contributing to fetal development. The objectives of the present study were to investigate the association between maternal glycemic status with fetal growth and to examine the role of *MEG3* DNA methylation profile in this association.

METHODS

Participants

GDM was diagnosed using a 75-g oral glucose tolerance testing (OGTT) carried out at 24–28 weeks" gestation, according to the International Association of Diabetes in Pregnancy Study Group criteria[25]. Women were diagnosed with GDM if at least one value exceeded the following thresholds: fasting plasma glucose ≥5.1 mmol/L, 1-h plasm glucose ≥10.0 mmol/L, 2-h plasma glucose ≥8.5 mmol/L[26] and recommended with dietary treatment. The glycated hemoglobin levels were also measured at the time of OGTT. We excluded women with pre-GDM, multifetation, hyperthyroidism, cardiovascular disease and pre-eclampsia. The gestational age-matched women with normal glucose tolerance were in the control group. Body mass index (BMI) was calculated according to the standard formula (kg/m^2) in the first trimester and before delivery.

The study was approved by the ethics committee of the Women's Hospital, School of Medicine, Zhejiang University (IRB-20200194-R), and followed the Declaration of Helsinki. Written informed consent was obtained from all participants.

Placental tissue sampling

Placenta samples were collected in the minutes after delivery (<30 min postpartum) by well-trained research staff, and kept in RNALater (Qiagen, Valencia, CA, USA) at −80°C until nucleic acid extraction. We selected tissues from both sides of the placenta, the maternal side consisted of the intervillous tissues and chorionic villi, and the fetal side consisted mainly of fetal villous tissue. Analyses were carried out on both sides independently.

RNA extraction and real-time polymerase chain reaction analyses

Total RNA was extracted from collected placenta tissues using the Trizol reagent (Invitrogen, Carlsbad, CA, USA), and then reverse-transcribed to complementary DNA with the prime-Script™ RT reagent Kit (TaKaRa, Otsu, Japan) according to the manufacturer's instructions, respectively. The quantitative real-time polymerase chain reaction (PCR) was carried out with SYBR® Premix Ex Taq™ (TaKaRa) in an Applied Biosystems 7900HT (ABI, Foster City, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase was chosen as the endogenous control, and the relative expression of *MEG3* was processed with the $2^{-\Delta\Delta C_t}$ method[27]. All the samples were run in triplicate. The primer sequences used are shown in Table S1.

DNA extraction and methylation-specific PCR

Genomic DNA was purified from placenta samples with the Genomic DNA Purification Kit (Invitrogen), and bisulfite conversion was carried out with the EpiTect Bisulfite Kit (Qiagen) according to the manufacturer's protocols. Regions of interest were carried out for multiplex and index PCR reaction, as described in a previous study[28]. The PCR amplicons were purified by the QIAquick Gel Extraction Kit (Qiagen) and loaded onto Illumina NextSeq 500 (Illumina, San Diego, CA, USA) according to the manufacturer's protocols. Specific primers for PCR amplification are presented in Table S2.

Statistical analysis

Statistical analyses were carried out using SPSS 25.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA, USA), and results were presented as the mean ± standard deviation. Differences in continuous variables among groups were carried out by the independent two-sample Student's *t*-test. Categorical variables are reported as numbers (percentages) and were compared by Fisher's exact test. Pearson correlation analysis was carried out to examine associations between placental *MEG3* methylation profile and clinical variables of interest: maternal glucose levels of OGTT and neonatal birthweight. The associated results were adjusted by multivariate linear regression using the enter method for maternal age, BMI (during the first and third trimester), height, gestational age and newborn sex. Differences were assigned statistically significant at $P < 0.05$, $P < 0.01$ or $P < 0.001$. The sample size was estimated to obtain a significant Pearson correlation coefficient of at least 0.4 between methylation levels and clinical variables. The total estimated sample size was 46 by accepting an alpha risk of 0.05 and a beta risk of 0.2.

RESULTS

Study participants

A total of 46 women with normal glucose tolerance ($n = 23$) or GDM ($n = 23$) participated in the present study. Descriptive statistics of mothers and newborns are presented in Table 1. The maternal characteristics, such as maternal age, height, BMI, gestational weeks and weight gain, were similar in the two groups. Meanwhile, fasting plasma glucose (4.31 ± 0.31 vs 4.91 ± 0.57 mmol/L, $P < 0.001$), 1-h plasma glucose (7.36 ± 1.32 vs 10.60 ± 1.84 mmol/L, $P < 0.001$), 2-h plasma glucose (6.65 ± 1.07 vs 9.37 ± 1.41 mmol/L, $P < 0.001$) and glycated hemoglobin (5.05 ± 0.26 vs 5.34 ± 0.43 (%), $P = 0.009$) concentrations were higher in the GDM group compared with the control group. Furthermore, the newborn birthweight of the GDM group was higher than the control group (3,178.91 ± 331.72 vs 3,541.30 ± 504.97 g, $P = 0.006$).

mRNA expression

To investigate the possible role of intrauterine hyperglycemia in the alteration of *MEG3* expression, we first assessed the mRNA expression of *MEG3* in the placenta using quantitative real-time PCR. As shown in Figure 1a, the mRNA expressions of *MEG3* on the maternal placental side were significantly reduced in the GDM patients compared with the healthy controls. Interestingly, this downregulation did not occur on the fetal side of the placenta (Figure 1b).

Methylation status of *MEG3*-DMR in placental tissues

Then, we analyzed the DNA methylation levels of the *MEG3*-DMR in both maternal and fetal sides of placental tissues. Overall, 35 CpG dinucleotides in the *MEG3*-DMR were the epigenotype. The individual CpG analyses are presented in Table S3. On the maternal side of the placenta, the DNA methylation levels at seven CpG islands' loci were significantly higher in the GDM group compared with the control group (Figure 2a); whereas on the fetal side, only single CpG site showed significant hypermethylation in the GDM group (Figure 2c). On average, the mean *MEG3* methylation levels on the maternal placental side were significantly different between the two groups (40.64 ± 2.15 vs 38.33 ± 2.92; $P = 0.004$; Figure 2b). However, there were no significant differences in mean *MEG3* methylation between women with or without GDM on the fetal side of the placenta. (39.87 ± 4.97 vs 38.36 ± 4.62; $P = 0.294$; Figure 2d).

Correlations between placental *MEG3* methylation levels, maternal hyperglycemia and fetal growth

Furthermore, we investigated whether the placental methylation status of the *MEG3*-DMR might be associated with maternal and

Table 1 | Characteristics of the mothers and newborns

Characteristics	Control ($n = 23$)	GDM ($n = 23$)	*P*-value
Maternal age (years)	31.87 ± 4.67	33.87 ± 4.85	0.161
Height (cm)	160.35 ± 6.51	158.48 ± 4.41	0.261
Gestational age at birth (weeks)	38.22 ± 1.17	38.04 ± 1.19	0.619
First trimester BMI (kg/m^2)	22.12 ± 3.32	23.20 ± 2.75	0.235
Third trimester BMI (kg/m^2)	27.25 ± 3.11	27.69 ± 2.78	0.618
Weight gain (%)[†]	24.02 ± 8.76	19.77 ± 7.46	0.083
Fasting glucose levels (mmol/L)	4.31 ± 0.31	4.91 ± 0.57	**<0.001**[*]
1-h OGTT glucose levels (mmol/L)	7.36 ± 1.32	10.60 ± 1.84	**<0.001**[*]
2-h OGTT glucose levels (mmol/L)	6.65 ± 1.07	9.37 ± 1.41	**<0.001**[*]
HbA1c (%)	5.05 ± 0.26	5.34 ± 0.43	**0.009**[*]
Birthweight (g)	3,178.91 ± 331.72	3,541.30 ± 504.97	**0.006**[*]
Newborn sex, male (%)	12 (52.2%)	14 (60.9%)	0.767

Data are presented as Mean ± SD and number (%).
[†]Weight gain between the first trimester and third trimester (% of initial body weight).
[*] $P < 0.05$ (in bold).

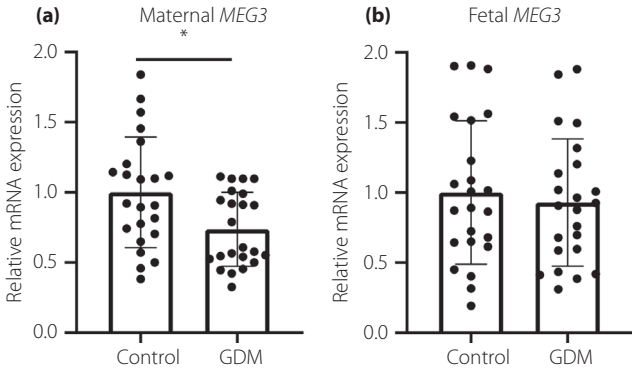

Figure 1 | Analysis of relative maternally expressed gene 3 (*MEG3*) messenger ribonucleic acid (mRNA) levels in gestational diabetes mellitus (GDM; $n = 23$) and control ($n = 23$) placentas. (a) On the maternal side of the placenta, *MEG3* expression was significantly downregulated in GDM patients compared with controls. (b) On the fetal side, there was no significant difference between GDM and control groups. Data are presented as the mean ± standard error; $^*P < 0.05$.

newborn characteristics. On the maternal side of the placenta, we found that the increasing level of maternal fasting glucose was positively correlated with methylation levels at five *MEG3* CpG islands (CpG9[chr14:101,292,225], CpG15[chr14:101,292,460], CpG16[chr14:101,292,463], CpG18[chr14:101,292,483] and CpG19[chr14:101,292,493]; data not shown). Furthermore, correlations were also observed between newborn birthweight and placental DNA methylation levels at five *MEG3* CpG islands (CpG9 [chr14:101,292,225], CpG11[chr14:101,292,392], CpG12 [chr14:101,292,398], CpG15[chr14:101,292,460] and CpG16 [chr14:101,292,463]; data not shown). Of note, the levels of *MEG3* DNA methylation on the maternal side at three specific CpG islands (CpG9, CpG15 and CpG16) were correlated with both maternal glucose status ($R = 0.447$, $P = 0.002$; $R = 0.461$, $P = 0.001$; $R = 0.579$, $P < 0.001$, respectively) and newborn birthweight ($R = 0.495$, $P < 0.001$; $R = 0.450$, $P = 0.001$; $R = 0.471$, $P = 0.001$, respectively; Figure 3). The correlations remained significant after adjusting for maternal age, height, BMI, gestational age and newborn sex as confounders by carrying out multivariable linear regression (Table 2). Furthermore, the

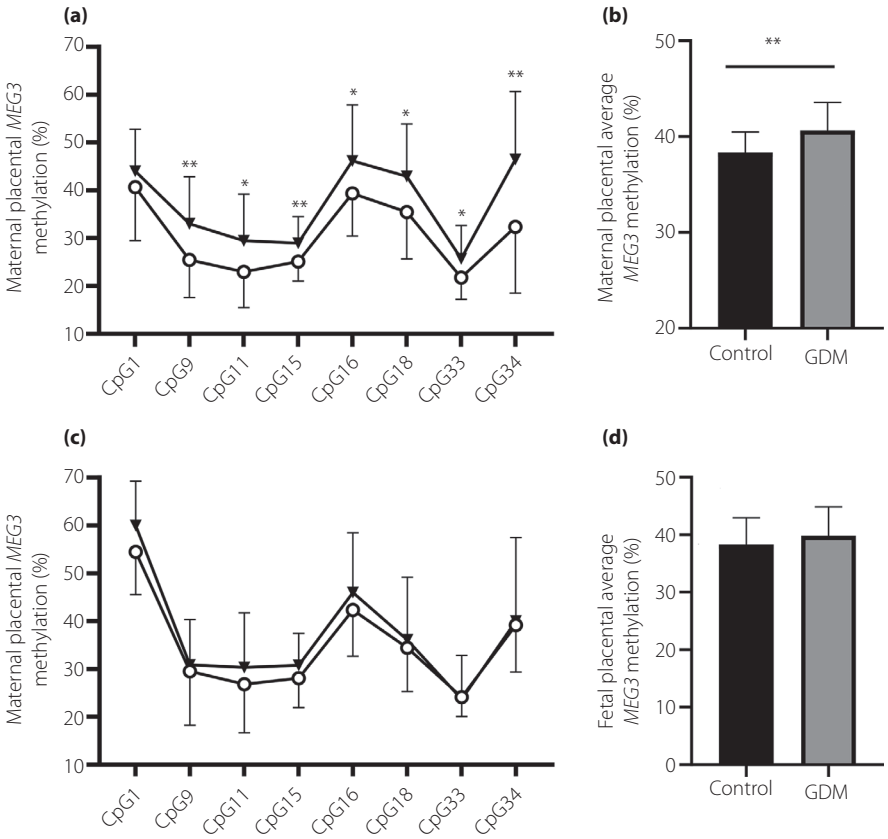

Figure 2 | Comparison of maternally expressed gene 3 (*MEG3*) deoxyribonucleic acid methylation levels in gestational diabetes mellitus (GDM; $n = 23$) and control ($n = 23$) placentas. (a,b) Methylation status of *MEG3* differentially methylated region (DMR) on the maternal side, including the (a) mean *MEG3* methylation level and (b) specific individual CpG sites. (c,d) *MEG3*-DMR methylation status on the fetal side, including the (c) mean *MEG3* methylation level and (d) specific individual CpG sites. GDM and the control group are indicated by triangles (▼) and circles (○), respectively. Data are presented as the mean ± standard error; $^*P < 0.05$; $^{**}P < 0.01$.

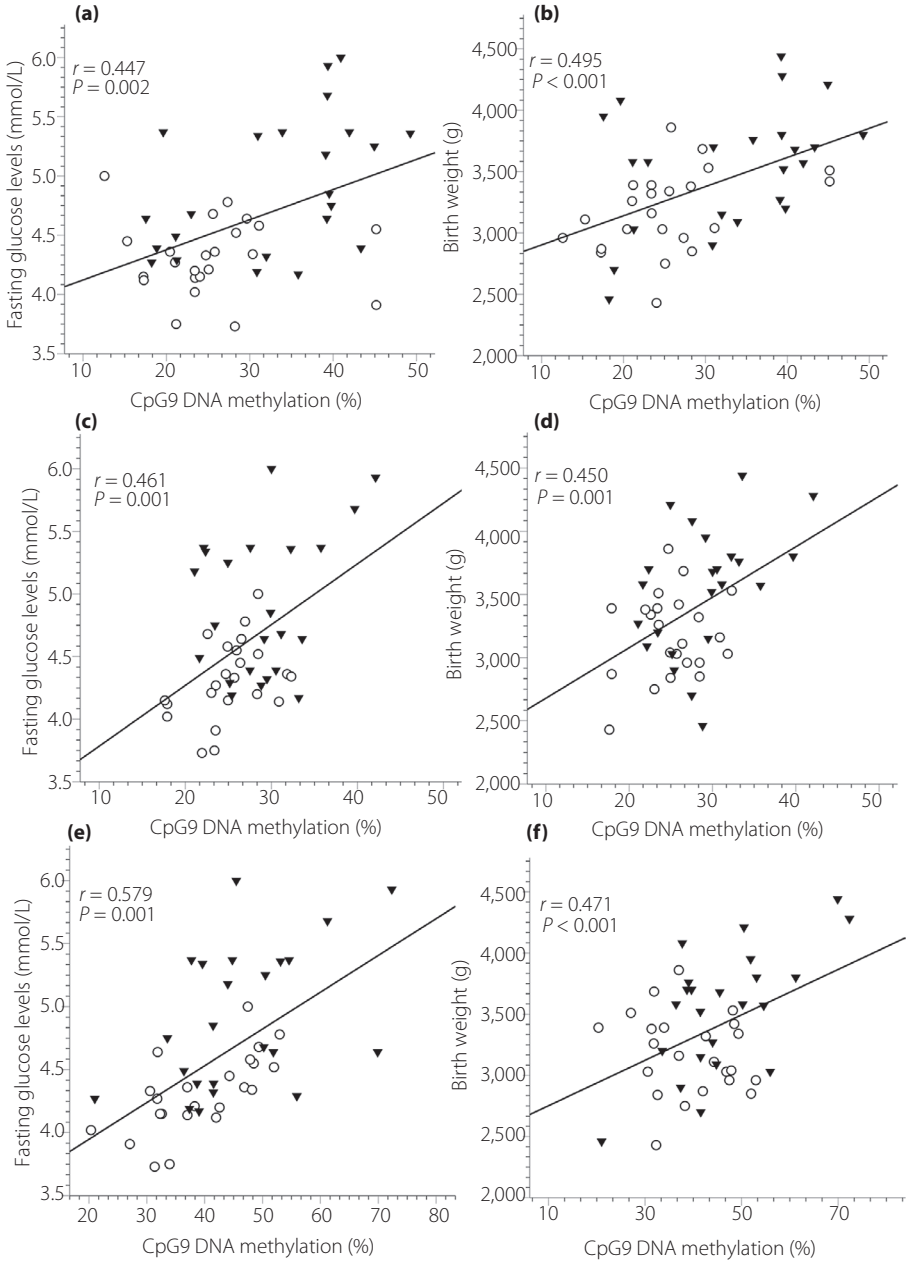

Figure 3 | Pearson correlation for the association between placental deoxyribonucleic acid (DNA) methylation levels at specific individual CpG sites in the maternally expressed gene 3 (*MEG3*) differentially methylated region (DMR) and maternal glucose levels and newborn birthweight. (a,b) The correlation between the *MEG3* methylation and (a) maternal fasting glucose levels and (b) newborn birthweight at CpG9, (c,d) at CpG15, and (e,f) at CpG16. GDM (n = 23) and the control (n = 23) group are indicated by triangles (▼) and circles (○), respectively.

higher mean methylation levels of the *MEG3*-DMR on the maternal side of the placenta also showed a significant correlation with higher maternal fasting glucose concentration (R = 0.603, P < 0.001) and newborn birthweight (R = 0.568, P < 0.001; Figure 4). These relationships substantially improved after adjusting for confounding variables (Table 2). It suggests that hypermethylation in the *MEG3*-DMR, especially at CpG9, CpG15 and CpG16, is related to intrauterine hyperglycemia and conceptus growth. However, there was no correlation between DNA methylation levels of the *MEG3*-DMR and the maternal glucose status or newborn birthweight on the fetal side of the placenta (data not shown).

DISCUSSION

The developmental origins hypothesis posits that prenatal environmental variations could disturb fetal programming, and lead

Table 2 | Multivariate linear models analyzing the correlation of maternally expressed gene 3 deoxyribonucleic acid methylation with selected clinical variables

	β	*P*	R^2
Mean methylation levels of *MEG3*-DMR (%)			
Fasting plasma glucose	0.491	0.001	0.488
Birthweight	0.547	0.002	0.478
Methylation level at CpG9			
Fasting plasma glucose	0.463	0.004	0.376
Birthweight	0.483	0.015	0.330
Methylation level at CpG15			
Fasting plasma glucose	0.482	0.005	0.274
Birthweight	0.612	0.003	0.285
Methylation level at CpG16			
Fasting plasma glucose	0.665	0.000	0.445
Birthweight	0.547	0.009	0.258

Models are adjusted for maternal age, height, body mass index, gestational age at delivery and newborn gender. *MEG3*-DMR, maternally expressed gene 3 differentially methylated region.

to a lifetime risk of obesity and chronic diseases[29,30]. In addition, growing clinical and epidemiological evidence recognizes that exposure to GDM is associated with large for gestational age and obesity in later life. To date, the exact molecular mechanism of developmental programming remains unknown, while epigenetic modification might elucidate the phenomenon. In the present study, we investigated maternal fasting glucose levels in the second trimester regarding neonatal birthweight, and evaluated the role of DNA methylation differences within the *MEG3*-DMR in this association. For the first time, we showed that the hypermethylation of *MEG3*-DMR on the maternal side of the placenta was associated with maternal hyperglycemia and neonatal birthweight. In a total of 35 CpG islands analyzed, we found hypermethylation of three specific CpG sites in the *MEG3*-DMR as the most potential candidate gene sites for further research. Interestingly, these phenomena were not found on the fetal side. These results might indicate that DNA methylation of the *MEG3*-DMR plays an essential role in response to intrauterine hyperglycemia, and this epigenetic alteration is a link to offspring development.

As a mediator of environmental and maternal inputs throughout pregnancy, the placenta is central to coordinating fetal development. The epigenetic alterations of the placenta responding to maternal and fetal signals can contribute to determining fetal growth potential and influence health outcomes throughout life. Studies in both humans and animal models have shown that the DNA methylation profile of the placenta is one of the critical mechanisms. A previous animal study found that dysregulation of methylation patterns can lead to adverse placenta morphology and birth outcome[31]. A wide array of environmental and maternal signals might alter gene methylation in the placenta, such as maternal depressed mood[32], maternal hyperglycemia[33], poor nutritional[34] or overnutrition status[35] and pre-eclampsia[36]. There is compelling evidence that DNA methylation dysregulation underlies the associations between adult diseases and intrauterine hyperglycemia. Genome-wide DNA methylation variation in the GDM-exposed placenta compared controls identified 1,708 methylation variable positions achieving significant difference[37]. Another placental epigenome-wide association study identified methylation at fiftee15n CpG islands that were associated with newborn birthweight[38]. Thus far, the focus regarding placental epigenetic marking progress in GDM was set on specific DNA methylation loci. Bouchard *et al.*[39] reported a significant correlation between maternal glycemic status and DNA methylation levels at the adiponectin gene in the placenta. This relationship could increase the risk of developing diabetes and obesity in the offspring. Another relevant study was carried out by Houde *et al.*[40], who found that the 2-h post-OGTT results are correlated with adenosine triphosphate-binding cassette transporter A1 DNA methylation levels of the placenta. Then, the

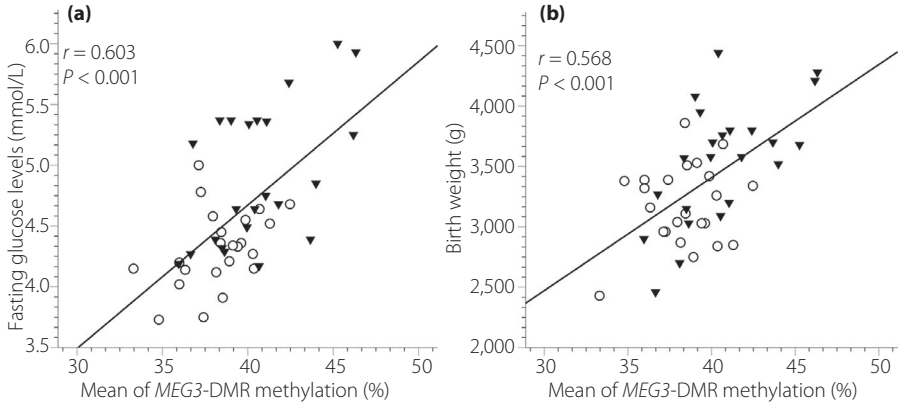

Figure 4 | Pearson correlation for the association between the mean placental deoxyribonucleic acid (DNA) methylation of maternally expressed gene 3 differentially methylated region (*MEG3*-DMR) and maternal fasting glucose levels and newborn birthweight. (a,b) Correlation between the (a) mean *MEG3*-DMR DNA methylation and fasting glucose levels and (b) newborn birthweight.

same authors found the association between maternal glycemia and placental lipoprotein lipase DNA methylation levels[13]. In addition, we added a novel finding that the hypermethylation status of the *MEG3*-DMR on the maternal side of the placenta is a link between maternal hyperglycemia and large neonatal birthweight. The variability of *MEG3*-DMR hypermethylation was only observed on the maternal placental side in the GDM group, and is perhaps a result of the intrauterine hyperglycemic environment.

Genomic imprinting is an epigenetic modification that causes unequal gene expression depending on their parental origin. It is one of the candidate mechanisms that contribute to regulating fetal growth in mammals. The animal model study by our team showed that intrauterine hyperglycemia could alter the expression of the imprinted genes of the placenta, 35 upregulated and 10 downregulated[41]. The study of the placenta exposed to intrauterine hyperglycemia suggested that altered methylation percentages at the multiple imprint regulatory regions, including the MEST and IGF2/H19, are associated with large for gestational age or macrosomia (birthweight >4 kg)[35,42]. Similar results were also identified in the children of obese mothers[43]. The present findings, together with the previous studies, support the idea that epigenetic modifications at some imprinted loci have been crucial regulators of both neonatal and long-term health outcomes.

MEG3 is a maternally expressed imprinting gene in humans, and produces a long non-coding RNA transcript[15]. The *MEG3* promoter region is rich in CpG islands and there are two DMRs – the IG-DMR the *MEG3*-DMR[20]. Previous studies showed that the methylation status of the *MEG3*-DMR plays an essential role in regulating the *MEG3* expression, which mediated cell proliferation, migration and apoptosis in pituitary tumor[34], retinoblastoma[11], cervical cancer[44], acute myeloid leukemia[45], and type 1 and 2 diabetes[19,46]. Mounting studies have reported that *MEG3* is involved in the progression of diabetes-related diseases. For example, Qiu *et al.*[47] found *MEG3* knockdown aggravated diabetes-related retinal microvascular dysfunction in mice by the inactivation of PI3k/Akt signaling. Additionally, *MEG3* protected against podocyte injury in diabetic nephropathy by regulating Wnt/β-catenin signaling[48]. Studies of patients with diabetes also suggested that increased methylation at the *MEG3*-DMR is sufficient to repress *MEG3* expression, and impacts the sensitivity of β-cells to cytokine-mediated oxidative stress[23]. However, the role of *MEG3* in fetal development and responding to the intrauterine hyperglycemic environment remains unknown. In the present study, we endeavored to show increased methylation levels of the *MEG3*-DMR in the GDM-exposed placenta, and whether the hypermethylation status is correlated with fetal growth. We showed that methylation levels in the *MEG3*-DMR of the placenta are positively correlated with newborn birthweight. Interestingly, a recent study carried out by Prats-Puig *et al.*[49] reported that placental *MEG3*-DMR methylation correlated negatively with length and weight gain during the first postnatal year. Similarly, a follow-up study also showed that children exposed to the diabetic environment *in utero* had a lower bodyweight at the age of 5 years than non-exposed children[50]. One possible explanation for this variation in rates of weight gain in the early postnatal is catch-up growth[51]. Another potential explanation is that GDM exposure might impact childhood growth; whether this relationship is mediated by epigenetic modification needs to be investigated.

The present study had several strengths. We analyzed the placental DNA methylation profile on the maternal and fetal side, independently. The hypermethylation of *MEG3*-DMR on the maternal side is strongly associated with maternal fasting glucose levels and newborn birthweight. Intriguingly, these relationships were not found on the fetal placental side. This supports the idea that maternal lifestyle is vital for the optimal development of the fetus[52]. Second, the individual CpG island of the *MEG3*-DMR was independently analyzed for the association between maternal glucose levels and birthweight. We found three specific sites to determine the relationship. Although our findings were generated confined to GDM patients, it is essential to realize that epigenetic modification of imprinting gene *MEG3* could be a hopeful mechanism involved in fetal programming in general ways. This altered DNA methylation markers might allow the early approach to assessing the prenatal or postnatal growth of offspring. Nevertheless, the present study had certain limitations. The number of participants might not be adequate, limiting the power of our study. In addition, a postnatal follow-up study should be carried out to show the impact of these epigenetic alterations on later-life health.

In conclusion, this is the first report showing that DNA methylation perturbations within the imprinted gene, *MEG3*-DMR, of the placenta are correlated with maternal glucose concentrations and newborn birthweight. Our investigations provide supporting evidence involved in fetal programming of obesity predisposition in adult life, underlining the consequences of adverse intrauterine exposures.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Primer list for quantitative real-time polymerase chain reaction.
Table S2 | Primer list for MethylTarget sequencing.
Table S3 | Methylation levels of individual CpG island in the maternally expressed gene 3 differentially methylated region of the placenta.