Placental DNA methylation changes in the development of gestational diabetes mellitus

Fujia Chen

Fudan University

Min Li

Fudan University

Xiaoping Fei

The First people's Hospital of Kunshan

Weiqiang Zhu

Fudan University

Zhaofeng Zhang

Fudan University

Yupei Shen

Fudan University

Yanyan Mao

Fudan University

Qianxi Zhu

Fudan University

Jianhua Xu

Fudan University

Weijin Zhou

Fudan University

Jing Du (✉ dujing42@126.com)

Fudan University

Research Article

Keywords: Gestational diabetes mellitus, DNA methylation, Placenta, TRIM67

Posted Date: April 5th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1375478/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Aims

The aim of this study was to investigate the association between gene methylation alterations and the development of GDM, as well as to investigate the potential epigenetic mechanisms of GDM.

Methods

Reduced representation bisulfite sequencing (RRBS) and RNA sequencing (RNA-seq) analysis were performed on placental tissues from women with GDM and healthy controls. After pyrosequencing, correlation and linear regression analysis were applied based on the effect of methylated-differentially expressed genes (DEGs).

Results

HOXD9, *ALDH1A2*, *CSMD1*, *TRIM67*, and *PAX8*, five candidate DEGs, were identified. *TRIM67* exhibited significant hypermethylation levels at different CpG sites. Hypermethylation of *TRIM67* promoter was significantly positively correlated with 1h and 2h OGTT glucose levels and negatively correlated with Lipoprotein(a).

Conclusions

TRIM67 hypermethylation in the placenta of women with GDM may correlate with insulin resistance, which may play a role in the development of GDM. These findings provide new insights for further studies on the pathophysiology of GDM.

1 Background

Gestational diabetes mellitus (GDM) has been defined as ‘any amount of glucose intolerance with an onset or first detection during the pregnancy period’ [1]. The prevalence of GDM among women of reproductive age has shown a significant year-on-year rise worldwide, ranging from < 1–28% [2]. The total incidence of GDM in mainland China is 14.8% [3].

Expectant mothers with advanced maternal age (≥ 35 years), overweight, hyperglycemia or family history of type 2 diabetes (T2DM), are likely to experience difficulties with managing insulin sensitivity [4, 5]. Women with GDM are at a heightened risk for developing chronic metabolic and cardiovascular injuries during maternity, and their offspring are more likely to develop obesity and T2DM during their lifetime [6]. Adverse pregnancy outcomes may include premature delivery, spontaneous abortion, and stillbirth [7]. Furthermore, GDM may have a long-term detrimental impact on both the mother and fetus, such as in the cases of fetal macrosomia, newborn hyperinsulinemia, neonatal hypoglycemia, maternal adiposis, and cardiovascular diseases [8, 9]. In fact, these risks are determined at the stage of embryonic and fetal growth in utero, an approach known as the Development Origins of Health and Disease (DOHaD) [8]. An abnormal intrauterine environment, resulting from maternal sub-health and excessive nutrient input contributes to increase susceptibility to developing metabolic diseases in the future [10].

The placenta is a pivotal organ of material exchange between the mother and fetus. The characteristics of GDM is glucose intolerance induced by intrauterine hyperglycemia which may lead to permanent placental epigenetic modification [11]. Epigenetic variations mediated by detrimental intrauterine environment is regarded as potential biomarkers to find a metabolic impact on the maternal endocrine regulation and fetal development [12]. It has been reported that placental DNA methylation is primarily related to the regulation of maternal insulin sensitivity [13]. A large-scale quantitative assessment confirmed placental DNA hypermethylation was associated with GDM, independent of established risk factors [14]. So far, increasing studies come to provide interaction between placental epigenetic variations and maternal glucose concentrations to verify underlying molecular pathophysiological mechanisms of GDM [15–17].

The aim of this study was to explore the possible role of placental DNA methylation in the development of GDM, as well as to investigate the potential epigenetic mechanisms of GDM.

2 Methods

2.1 Sample Collection

The original case-control study enrolled a total of 2803 singleton pregnant women, including 249 women with GDM and 2554 healthy women from the First People's Hospital of Kunshan, Jiangsu Province, China, from July 2016 to December 2016. The standard of diagnosis for GDM

during gestational weeks 24 and 28 included the fasting plasma glucose > 5.1 mmol/l, one-hour oral glucose tolerance test (OGTT) > 10 mmol/l, or two-hour OGTT > 8.5 mmol/l, according to the criteria of International Association of Diabetes and Pregnancy Study Groups (IADPSG) [18]. Women whose glucose levels met any of the OGTT values were assigned to the GDM group. Women who had no history of GDM or diabetes and had normal glucose tolerance during pregnancy were assigned to the healthy group. Women with severe cardiovascular diseases, pre-existing diabetes mellitus related to glucose metabolism disorders, or other complicated diseases were excluded.

A total of 24 healthy women were randomly selected and matched according to the age at delivery of 24 women with GDM. Human placenta samples (N = 48) of pregnant women were obtained within two hours after delivery for subsequent analysis. Placenta samples were immediately frozen in liquid nitrogen at -80°C for further analysis. The ethics committee of Shanghai Institute of Planned Parenthood Research approved this study (Number: PJ2016-16), and all study participants provided written informed consent in accordance with the Declaration of Helsinki.

2.2 Reduced representation bisulfite sequencing (RRBS)

Total genomic DNA was extracted from placental samples using an EasyPure® Genomic DNA Kit according to the manufacturer's instructions. Genome-wide DNA methylation profiles from five GDM samples and five healthy samples were examined via RRBS. Ten samples used for RRBS analysis were randomly selected from placental samples (N = 48). Briefly, 500 ng of extracted DNA was digested using the MspI enzyme. DNA fragments were processed by 3' end-repair, A' tailing and methylated adaptor ligation. After bisulfite disposal, PCR amplification was followed. Multiplexed samples were pooled into a DNA library and 100 bp paired-end sequenced (HiSeq-2000, Illumina).

2.3 RNA sequencing

Total RNA was isolated using the EasyPure RNA Kit according to the manufacturer's protocols. RNA-Seq was performed on the same five GDM samples and five healthy samples as RRBS on an Illumina HiSeq-2000 platform with 150 bp paired-end reads according to Illumina's protocol. p -Value < 0.05 and $|\log_2[\text{fold change (FC)}]| > 0.8$ were assigned as a threshold for differentially expressed genes (DEGs).

2.4 Functional and pathway enrichment analysis

Gene Ontology (GO) was conducted using the Gene Ontology Consortium (www.geneontology.org/), and pathway enrichment analysis of GDM was performed according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database.

2.5 Pyrosequencing for quantifying DEGs

Significant DEGs ([false discovery rate (FDR)] p -Value < 0.05) were evaluated using a pyrosequencing assay. Extracted DNA from totally 48 human placental samples was conducted using the QiagenEpiTect Bisulfite Kit (Qiagen, 59104). The primers were designed using PyroMark Assay Design 2.0, and the sequences used for the verification cohort are shown in Table S1. PCR amplification (ABI 9700 PCR System, Applied Biosystems) was used for 40 circles. Pyrosequencing was performed using the PyroMark Q96/48 ID (Qiagen), and the final data were calculated using the PyroQ CpG software (Biotage) according to the manufacturer's instructions.

2.6 Statistical analysis

Statistical analyses and graph generation were performed using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA), R statistical software and Prism 8 (GraphPad Software Inc.). A two-sided test was performed with a p -Value < 0.05 being the threshold for statistical significance. Continuous data were presented as mean $\pm$ standard deviation (SD). Categorical data were presented as frequencies (percentages). Differences between the GDM and healthy groups were evaluated using the independent samples t -test for continuous variables with normal distribution, and the Pearson chi-square test for categorical variables. Spearman's correlation coefficients were used to assess the association between placenta DNA methylation and blood glucose and lipid concentrations.

3 Results

3.1 Clinical patient characteristics

The characteristics of the 48 Chinese participants (aged 20–40 years) and their newborns in the study are shown in Table 1. The majority of women with GDM had normal body mass index (BMI) before pregnancy (54.17%), more than a high school education (58.33%), normal prenatal systolic pressure (95.83%) and diastolic pressure (83.33%), in addition to healthy controls (83.33%, 70.84%, 100% and 100%, respectively). Women with GDM had significantly higher fasting (p -Value = 0.002), 1h oral glucose tolerance test (OGTT), and 2h OGTT (p -Value < 0.001) glucose concentrations compared to women without GDM.

Table 1
Participants' characteristics in the study (N = 48)

Variables	GDM(n = 24)	Control(n = 24)	p-Value
	Mean ± SD or n(%)		
Maternal age (years) ^a	28.50 ± 2.15	27.29 ± 4.42	0.237
Pre-pregnancy BMI (kg/m ²) ^a	22.21 ± 3.98	20.87 ± 2.18	0.154
Education ^b			0.219
<high school	0	2(8.33)	
high school or equivalent	10(41.67)	5(20.83)	
> high school	14(58.33)	17(70.84)	
Prenatal systolic pressure (mmHg) ^a	113.83 ± 12.25	114.17 ± 10.26	0.919
Prenatal diastolic pressure (mmHg) ^a	68.54 ± 7.81	70.83 ± 6.93	0.288
Fasting glucose (mmol/L) ^a	4.79 ± 0.62	4.33 ± 0.29	0.002
1hr OGTT (mmol/L) ^a	9.69 ± 1.58	7.40 ± 1.21	< 0.001
2hr OGTT (mmol/L) ^a	8.26 ± 2.07	5.81 ± 1.11	< 0.001
TC (mmol/L) ^a	6.04 ± 1.14	6.08 ± 0.99	0.895
TG (mmol/L) ^a	3.34 ± 1.33	3.75 ± 1.74	0.365
HDL (mmol/L) ^a	1.88 ± 0.29	1.77 ± 0.34	0.214
LDL (mmol/L) ^a	3.60 ± 1.31	3.59 ± 0.97	0.994
ApoA1 (g/L) ^a	1.70 ± 0.21	1.66 ± 0.20	0.501
ApoB (g/L) ^a	1.17 ± 0.32	1.16 ± 0.22	0.860
Lp(a) (mg/L) ^a	179.09 ± 175.07	198.13 ± 219.12	0.744
Gestational age at delivery (weeks) ^a	39.40 ± 0.90	39.77 ± 0.90	0.156
Infant sex ^b			0.146
Boy	13(54.17)	8(33.33)	
Girl	11(45.83)	16(66.67)	
Birth weight (g) ^a	3462.50 ± 421.51	3287.92 ± 270.77	0.095
Pearson Chi-Square tests for categorical variables, and independent-samples t-test for continuous variables. Data are expressed as ^a mean ± standard error of the mean, or as ^b count (percentage).			
Abbreviation: GDM, gestational diabetes mellitus; BMI, body mass index; FG, fasting glucose; OGTT, oral glucose tolerance test; TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; ApoA1, Apolipoprotein A1; ApoB, Apolipoprotein B; Lp(a), Lipoprotein (a).			

3.2 Identification of placental DMGs and DEGs between GDM and healthy groups

In total, 14374 CpG sites in promoter region were identified by RRBS analysis. 4224 hypomethylated CpG sites of 2593 genes and 3348 hypermethylated CpG sites of 2166 genes were screened in GDM placental samples compared to healthy groups (p -Value < 0.01). Every differentially methylated CpG site, from 22 autosomal chromosomes, was recorded in the circus plot (Fig. 1). The proportion of all CpG sites on the genomic location and differentially methylated CpG sites are shown in Fig. 2A. All differentially expressed genes including up-regulated and down-regulated genes are presented in the volcano plot (Fig. 2B).

Totally 16473 transcripts were acquired. Based on the screening limitation (p -Value < 0.05 and $|\log_2FC| > 0.8$), 269 DEGs from GDM placental samples were identified compared to healthy ones, with 81 significantly highly expressed genes and 188 genes with low expression. For 5

hypomethylated-highly-expressed genes and 19 hypermethylated genes with low expression, the overlapping aberrant methylated genes and regulated genes are shown in the Venn graph (Fig. 3A, 3B). Due to degradation in a pair of RNA samples, we conducted RNA-seq using four GDM samples and four healthy controls. The heatmap indicates the expression levels of DEGs from the eight samples (Fig. 3C), including *VSTM1*, *SYT6*, *PRSS46*, *HAS1*, *CSMD1*, *ZNF774*, *UTS2B*, *TSHZ2*, *TRIM67*, *TMEM246*, *SCNN1A*, *PTGER1*, *PAX8*, *LY6D*, *ISLR2*, *HOXD9*, *HOXA4*, *GREB1L*, *C9orf129*, *C1QL1*, *APOA1*, *ALOX15*, *ALDH1A2*, and *ACP5*. The locations of the aforementioned 24 DEGs on the chromosome are indicated with yellow and green fonts in Fig. 1.

Comparing the other GDM-related DEGs, *HOXD9*, *ALDH1A2*, *CSMD1*, *TRIM67* and *PAX8* (Table 2), five candidate genes from placental samples (FDR *p*-Value < 0.05), were analyzed via bisulfite pyrosequencing. Moreover, CpG sites 3, 5, 7 and 9 (Chromosome 1: 231161412–231161463) in *TRIM67* exhibited significant hypermethylation levels in GDM samples compared to healthy controls (Fig. 4). The methylation levels of CpG sites in other four candidate genes with no statistic difference were shown in Figure S1.

Table 2
Basic information for the top five candidate differentially expressed genes between 24 GDM samples and 24 healthy controls.

ID	Genes	log ₂ FC	t	B	Location	p-Value	Expression
ENSG00000128709	HOXD9	3.3599	2.5548	-4.2906	Chr2:176122719–176124937	0.0331	Down
ENSG00000119283	TRIM67	2.5555	2.7175	-4.2606	Chr1:231162058–231221565	0.0257	Down
ENSG00000128918	ALDH1A2	2.2201	0.8034	-4.6246	Chr15:57953429–58065711	0.041	Down
ENSG00000183117	CSMD1	-2.425	-1.3792	-4.5472	Chr8:2935353–4995035	0.027	Up
ENSG00000125618	PAX8	1.3525	2.457	-4.3092	Chr2:113215997–113278921	0.0387	Down

3.3 Functional and pathway enrichment analysis

GO functional analysis showed that most DEGs were involved in biological processes, such as immune response to stimulus, biological regulation, and metabolic processes (Fig. 5). KEGG enrichment analysis indicated that they were related to antigen processing and presentation, intestinal immune network for IgA production, and type I diabetes mellitus in Fig. 6.

3.4 Regression and correlation analysis to identify the association between the methylation levels and glucose/lipid level of GDM

Table 3 and Table S2 presents the associations between glucose/lipid level of GDM and placental DNA methylation changes in *TRIM67* CpG sites. The methylation levels of *TRIM67* CpG Site3 and Site7 were significantly positively correlated with 1h OGTT (*p*-Value = 0.001) and 2h OGTT (*p*-Value = 0.024, 0.005, respectively). DNA methylation at position 5 of *TRIM67* was significantly positively associated with 1h OGTT (*p*-Value < 0.001) and 2h OGTT (*p*-Value = 0.002), while negatively correlated with Lipoprotein(a) [Lp(a)] (*p*-Value = 0.01). Furthermore, the Site9 of *TRIM67* showed an inverse correlation with Lp(a) with a statistical significance (*p*-Value = 0.002).

Table 3
Spearman's correlation analysis showing the association between differentially methylated CpG sites of *TRIM67* and fasting glucose, 1 h OGTT, 2 h OGTT, and Lp(a) between 24 GDM samples and 24 healthy controls.

<i>TRIM67</i> CpG sites	FG(mmol/L)		1h OGTT(mmol/L)		2h OGTT(mmol/L)		Lp(a)(mg/L)	
	Rho	p-Value	Rho	p-Value	Rho	p-Value	Rho	p-Value
Site3	0.154	0.295	0.448	0.001	0.325	0.024	-0.284	0.053
Site5	0.241	0.098	0.565	< 0.001	0.427	0.002	-0.372	0.01
Site7	0.24	0.101	0.465	0.001	0.396	0.005	-0.219	0.139
Site9	0.209	0.153	0.266	0.067	0.216	0.141	-0.443	0.002
Spearman's correlation coefficient (Rho) is shown with significance at p < 0.05.								
Abbreviation: FG, fasting glucose; OGTT, oral glucose tolerance test; Lp(a), Lipoprotein (a).								

To estimate the degree of glucose/lipid metabolic effects by *TRIM67* CpG sites hypermethylation, the linear regression analysis was performed (Table 4). The significant correlations about the methylation of *TRIM67* Site5 with FG and 1h OGTT, *TRIM67* Site7 with 2h OGTT, and *TRIM67* Site9 with Lp(a) were observed.

Table 4

Linear regression analysis of differentially methylated TRIM67 CpG sites and glucose/lipid metabolism, adjusting for age and BMI between 24 GDM samples and 24 healthy controls.

Variables	FG (mmol/L)		1h OGTT (mmol/L)		2h OGTT (mmol/L)		Lp(a) (mg/L)	
	β (95%CI)	<i>p</i> -Value	β (95%CI)	<i>p</i> -Value	β (95%CI)	<i>p</i> -Value	β (95%CI)	<i>p</i> -Value
Site3	-0.017(-0.063;0.029)	0.460	0.020(-0.118;0.158)	0.768	0.001(-0.169;0.171)	0.994	-12.31(-28.75;4.13)	0.138
Site5	0.022(0.001;0.043)	0.042	0.193(0.051;0.336)	0.009	0.099(-0.076;0.274)	0.261	1.33(-15.03;17.69)	0.870
Site7	-0.001(-0.054;0.051)	0.962	-0.009(-0.168;0.150)	0.913	0.125(0.034;0.215)	0.008	15.29(-2.96;33.54)	0.098
Site9	0.023(-0.014;0.059)	0.211	-0.034(-0.144;0.076)	0.537	-0.052(-0.187;0.084)	0.447	-16.27(-29.05;-3.49)	0.014
Age	0.027(-0.017;0.071)	0.223	0.038(-0.094;0.171)	0.562	0.129(-0.035;0.292)	0.119	7.41(-8.29;23.11)	0.346
BMI	0.037(-0.012;0.087)	0.136	0.179(0.029;0.328)	0.020	0.107(-0.077;0.292)	0.245	-5.733(-23.02;11.55)	0.506

4 Discussion

In this study, 4224 hypomethylated CpG sites of 2593 genes and 3348 hypermethylated CpG sites of 2166 genes were identified. 269 DEGs with 81 significantly highly expressed and 188 genes with low expression were found in GDM placenta. GO and KEGG analysis showed majority of these DEGs functioned in metabolic processes and immune regulation processes. Several CpG sites in the promoter regions of *TRIM67* showed significant hypermethylation levels in GDM placentas compared with healthy placentas (*p*-Value < 0.05). We hypothesize that placental DNA methylation plays a role in the development of GDM. Further regression analysis found that *TRIM67* promoter hypermethylation may contribute to GDM processes, manifesting a significantly positive correlation with 1h and 2h OGTT glucose levels as well as a remarkably negative correlation with Lp(a).

The advantage of the current study is that it links between epigenetic alterations and the development of GDM to advocate more attention for maternal health such as body weight, blood glucose, and blood fat during pregnant periods and reflects the potential importance of methylation changes in the pathophysiology of GDM.

The pathophysiology of GDM is complex and involves endogenous, environmental, and genetic factors. Increasing studies have found that some alterations in placental DNA methylation may potentially regulate maternal insulin sensitivity and GDM risk [17, 19, 20]. It is well accepted that the insulin resistance is the initiator of GDM [21]. There exists insulin resistance in every pregnant woman to ensure continuous nutrient supply for fetus [22]. GDM is characterized by intrauterine hyperglycemia. It is also proposed that chronic hyperglycemia aggravate insulin resistance and reduce insulin sensitivity, especially in pregnancy [23]. In most cases, this hyperglycemia is the result of impaired glucose tolerance during pregnancy on a background of insulin resistance [24]. Moreover, inflammatory modulators may play an important role in the progression of GDM. It is reported that concentrations of C-reactive protein, TNF- α , and IL-6 elevate in women with GDM [25]. Higher levels of M1 macrophage and lower M2 macrophage were observed in GDM placenta in a recent case-control study [21]. Chronic inflammation and immune activation may play a crucial role in the development of T2DM and GDM [26]. However, the underlying cellular processes of GDM are yet to be elucidated.

The tripartite motif (TRIM) family, a major subset of the really interesting new gene (RING)-finger domain E3 ubiquitin ligases (≥ 80 members in humans), functions by adjusting biological processes of innate immune responses [27, 28]. Ubiquitination, catalyzed by E3 ubiquitin ligases and other enzymes, is a post-translational modification mechanism, that plays an important role in regulating innate immune signaling pathways in eukaryotes [29, 30]. As a subclass I TRIM family member, TRIM67 is expressed during embryonic development [31] and has E3 ubiquitin ligase activity, characterized by an N-terminal RING-finger domain, two zinc-finger domains (B1, B-box domain 1; B2, B-box domain 2), a coiled-coil multimerization region, as well as a C-terminal COS domain, fibronectin type III repeat (FNIII), and SPRY domain [32]. Overexpression of TRIM67 elevated the expression of p52 but reduced p100 (NF- κ B2 precursor) levels, indicating that *TRIM67* expression could promote NF- κ B2 gene processing, a non-canonical pathway of NF- κ B pathways [33]. This NF- κ B pathway is involved in multiple metabolic diseases including obesity and T2DM, which are associated with both dysregulated immune responses and chronic inflammation [34, 35]. A possible mechanism for the development of GDM may be mediated by hypermethylated *TRIM67* through inflammatory signaling.

Our findings are the first to present a significant epigenetic regulation of *TRIM67* in the development of GDM and a significant association of *TRIM67* methylation changes with levels of OGTT glucose levels and Lp(a). It is well established that gestational fasting and 2h OGTT glucose levels are highly correlated with risk of progression to diabetes [36]. Elevated glucose provides more possibilities to activate inflammation response signaling in diabetic complications [37]. Furthermore, evidences have also showed that Lp(a) have an inverse association with risk of diabetes and insulin resistance, with a lower level observed in T2DM cases [38–40]. That is, Lp(a) may be a less strong metabolic indicator in

the course of insulin resistance [40]. Through regression analysis, we further validated consistent findings among GDM patients, which supported the possibility that DNA methylation of some placental genes were altered intensely under adverse intrauterine exposures [41]. Methylated-differentially expressed genes could potentially be used as epigenetic biomarkers for the early diagnosis of GDM. In a word, our study preliminarily identified that DNA methylation changes of *TRIM67* may be one of the causative agents of GDM development and that its underlying biological drivers may be related to inflammation and oxidative stress.

The current study has a few limitations. First, larger placental sample sizes are needed, and maternal/cord blood would be considered for further experimental validation to compare pre-pregnancy with post-pregnancy to establish an explicit causal link between epigenetic alterations and glucose/lipid metabolic programming. Second, we roughly calculated the mean levels of blood glucose and lipid of participants, wherein some unmeasured variables including dietary habits, individual smoking or second-hand smoking status, indoor air quality, and occupational history of each pregnant woman were potentially confounders. Third, the mixture of various cells in placenta is also a limitation to this study, further research is trying to include single cell analysis especially in placental tissues whenever possible. Thus, in the future studies, more detailed variations with the potential inflammatory pathways to affect the development of GDM should be considered.

5 Conclusions

This preliminary study suggests that the level of DNA methylation changed in the placenta of GDM. Especially, the hypermethylation of *TRIM67* in GDM women may correlate with insulin resistance, which may play a role in the development of GDM. These findings provide new insights for further studies on the pathophysiology of GDM.

Declarations

Ethics approval and consent to participate

The ethics committee of Shanghai Institute of Planned Parenthood Research approved this study (Number: PJ2016-16), and all study participants provided written informed consent in accordance with the Declaration of Helsinki.

Consent for publication

Not available

Competing interest

The authors declare that they have no conflicts of interests.

Funding

This work was supported by Natural Science Foundation of Shanghai [grant number: 19ZR1444700], Projects of Shanghai Municipal Health Commission [grant number: 201940405], Open Foundation of Key Laboratory of Birth Regulation and Control Technology of National Health Commission of China [grant number: 2021003], and Program of Shanghai Municipal Health Commission [grant number: 20214Y0331].

Availability of data and materials

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request

Author contribution

Fujia Chen: Methodology, Validation, Formal analysis, Writing - Original Draft.

Min Li: Methodology, Funding acquisition, Project administration, Writing - review & editing.

Xiaoping Fei: Methodology, Resources, Investigation.

WeiqliangZhu: Methodology, Software, Visualization, Writing - review & editing.

Zhaofeng Zhang: Methodology, Validation, Formal analysis.

Yupei Shen: Methodology, Funding acquisition, Writing - review & editing.

Yanyan Mao: Methodology, Writing - review & editing.

Qianxi Zhu: Methodology, Writing - review & editing.

Jianhua Xu: Methodology, Funding acquisition, Writing - review & editing.

Weijin Zhou: Methodology, Writing - review & editing.

Jing Du: Methodology, Funding acquisition, Project administration, Writing - review & editing.

All authors have approved the final article.

Acknowledgments

We sincerely thank all participants in the study.

References

1. Radzicka S, Pietryga M, Iciek R, et al. The role of visfatin in pathogenesis of gestational diabetes (GDM). *Ginekol Pol.* 2018;89(9): 518–521. doi: 10.5603/GPa2018.0088.
2. Wong V, Lin A and Russell H. Adopting the new World Health Organization diagnostic criteria for gestational diabetes: How the prevalence changes in a high-risk region in Australia. *Diabetes Res Clin Pract.* 2017;129(2): 148–153. doi: 10.1016/j.diabres.2017.04.018.
3. Gao C, Sun X, Lu L, et al. Prevalence of gestational diabetes mellitus in mainland China: A systematic review and meta-analysis. *J Diabetes Investig.* 2019;10(1): 154–162. doi: 10.1111/jdi.12854.
4. Plows J, Stanley J, Baker P, et al. The Pathophysiology of Gestational Diabetes Mellitus. *Int J Mol Sci.* 2018;19(11): 3342–3363. doi: 10.3390/ijms19113342.
5. Bashir M, Aboulfotouh M, Dabbous Z, et al. Metformin-treated-GDM has lower risk of macrosomia compared to diet-treated GDM- a retrospective cohort study. *J Matern Fetal Neonatal Med.* 2020;33(14): 2366–2371. doi: 10.1080/14767058.2018.1550480.
6. Yoffe L, Polsky A, Gilam A, et al. Early diagnosis of gestational diabetes mellitus using circulating microRNAs. *Eur J Endocrinol.* 2019;181(5): 565–577. doi: 10.1530/eje-19-0206.
7. Stacey T, Tennant P, McCowan L, et al. Gestational diabetes and the risk of late stillbirth: a case-control study from England, UK. *Bjog.* 2019;126(8): 973–982. doi: 10.1111/1471-0528.15659.
8. Rogers L M, Serezani C H, Eastman A J, et al. Palmitate induces apoptotic cell death and inflammasome activation in human placental macrophages. *Placenta.* 2020;90(12): 45–51. doi: 10.1016/j.placenta.2019.12.009.
9. Barke T L, Goldstein J A, Sundermann A C, et al. Gestational diabetes mellitus is associated with increased CD163 expression and iron storage in the placenta. *Am J Reprod Immunol.* 2018;80(4): e13020-e13043. doi: 10.1111/aji.13020.
10. Chen P Y, Chu A, Liao W W, et al. Prenatal Growth Patterns and Birthweight Are Associated With Differential DNA Methylation and Gene Expression of Cardiometabolic Risk Genes in Human Placentas: A Discovery-Based Approach. *Reprod Sci.* 2018;25(4): 523–539. doi: 10.1177/1933719117716779.
11. Lesseur C and Chen J. Adverse Maternal Metabolic Intrauterine Environment and Placental Epigenetics: Implications for Fetal Metabolic Programming. *Curr Environ Health Rep.* 2018;5(4): 531–543. doi: 10.1007/s40572-018-0217-9.
12. Haertle L, El Hajj N, Dittrich M, et al. Epigenetic signatures of gestational diabetes mellitus on cord blood methylation. *Clin Epigenetics.* 2017;9(1): 28–30. doi: 10.1186/s13148-017-0329-3.
13. Hivert M F, Cardenas A, Allard C, et al. Interplay of Placental DNA Methylation and Maternal Insulin Sensitivity in Pregnancy. *Diabetes.* 2020;69(3): 484–492. doi: 10.2337/db19-0798.
14. Reichetzeder C, Dwi Putra S E, Pfab T, et al. Increased global placental DNA methylation levels are associated with gestational diabetes. *Clin Epigenetics.* 2016;8(1): 82–93. doi: 10.1186/s13148-016-0247-9.
15. Cheng C, Jiang Y, Yan T, et al. Placental MEG3-DMR methylation profile is associated with maternal glucose concentration and newborn birth weight. *J Diabetes Investig.* 2020;12(6): 432–445. doi: 10.1111/jdi.13432.
16. Gagné-Ouellet V, Houde A A, Guay S P, et al. Placental lipoprotein lipase DNA methylation alterations are associated with gestational diabetes and body composition at 5 years of age. *Epigenetics.* 2017;12(8): 616–625. doi: 10.1080/15592294.2017.1322254.
17. Xie X, Gao H, Zeng W, et al. Placental DNA methylation of peroxisome-proliferator-activated receptor- γ co-activator-1 α promoter is associated with maternal gestational glucose level. *Clin Sci (Lond).* 2015;129(4): 385–394. doi: 10.1042/cs20140688.
18. Tarnowski M, Malinowski D, Safranow K, et al. MTNR1A and MTNR1B gene polymorphisms in women with gestational diabetes. *Gynecol Endocrinol.* 2017;33(5): 395–398. doi: 10.1080/09513590.2016.1276556.

19. Chen M, Yan J, Han Q, et al. Identification of hub-methylated differentially expressed genes in patients with gestational diabetes mellitus by multi-omic WGCNA basing epigenome-wide and transcriptome-wide profiling. *J Cell Biochem.* 2020;121(5–6): 3173–3184. doi: 10.1002/jcb.29584.
20. Awamleh Z, Butcher D T, Hanley A, et al. Exposure to Gestational Diabetes Mellitus (GDM) alters DNA methylation in placenta and fetal cord blood. *Diabetes Res Clin Pract.* 2021;174(1): 108690–108702. doi: 10.1016/j.diabres.2021.108690.
21. Pan X, Jin X, Wang J, et al. Placenta inflammation is closely associated with gestational diabetes mellitus. *Am J Transl Res.* 2021;13(5): 4068–4079.
22. Teng Y, Xuan S, Jiang M, et al. Expression of H(2)S in Gestational Diabetes Mellitus and Correlation Analysis with Inflammatory Markers IL-6 and TNF- α . *J Diabetes Res.* 2020;2020(1): 1–11. doi: 10.1155/2020/3085840.
23. Lee D, Kim Y M, Jung K, et al. Alpha-Mangostin Improves Insulin Secretion and Protects INS-1 Cells from Streptozotocin-Induced Damage. *Int J Mol Sci.* 2018;19(5):1484–1497. doi: 10.3390/ijms19051484.
24. Jayawardane A, Patabendige M, Samaranyake D, et al. Hyperglycemia in pregnancy among South Asian women: A single tertiary care center experience from Colombo, Sri Lanka. *Diabetes Res Clin Pract.* 2018;145(1): 138–145. doi: 10.1016/j.diabres.2018.02.029.
25. Dasanayake A P, Chhun N, Tanner A C, et al. Periodontal pathogens and gestational diabetes mellitus. *J Dent Res.* 2008;87(4): 328–333. doi: 10.1177/154405910808700421.
26. Esser N, Legrand-Poels S, Piette J, et al. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res Clin Pract.* 2014;105(2): 141–150. doi:10.1016/j.diabres.2014.04.006.
27. Esposito D, Koliopoulos M G and Rittinger K. Structural determinants of TRIM protein function. *Biochem Soc Trans.* 2017;45(1): 183–191. doi: 10.1042/bst20160325.
28. Yang J, Fu Z, Zhang X, et al. TREM2 ectodomain and its soluble form in Alzheimer's disease. *J Neuroinflammation.* 2020;17(1): 204–210. doi: 10.1186/s12974-020-01878-2.
29. Yang W, Gu Z, Zhang H, et al. To TRIM the Immunity: From Innate to Adaptive Immunity. *Front Immunol.* 2020;11(1): 02157–02168. doi:10.3389/fimmu.2020.02157.
30. Wang H T and Hur S. Substrate recognition by TRIM and TRIM-like proteins in innate immunity. *Semin Cell Dev Biol.* 2021;111(1): 76–85. doi: 10.1016/j.semcdb.2020.09.013.
31. Meroni G. TRIM E3 Ubiquitin Ligases in Rare Genetic Disorders. *Adv Exp Med Biol.* 2020;1233(1): 311–325. doi: 10.1007/978-3-030-38266-7_14.
32. Hatakeyama S. TRIM Family Proteins: Roles in Autophagy, Immunity, and Carcinogenesis. *Trends Biochem Sci.* 2017;42(4): 297–311. doi: 10.1016/j.tibs.2017.01.002.
33. Liu R, Chen Y, Shou T, et al. TRIM67 promotes NF- κ B pathway and cell apoptosis in GA-13315-treated lung cancer cells. *Mol Med Rep.* 2019;20(3): 2936–2944. doi: 10.3892/mmr.2019.10509.
34. Baker R G, Hayden M S and Ghosh S. NF- κ B, inflammation, and metabolic disease. *Cell Metab.* 2011;13(1): 11–22. doi: 10.1016/j.cmet.2010.12.008.
35. Sun S C. The non-canonical NF- κ B pathway in immunity and inflammation. *Nat Rev Immunol.* 2017;17(9): 545–558. doi: 10.1038/nri.2017.52.
36. Kitabchi A E, Tempresa M, Knowler W C, et al. Role of insulin secretion and sensitivity in the evolution of type 2 diabetes in the diabetes prevention program: effects of lifestyle intervention and metformin. *Diabetes.* 2005;54(8): 2404–2414. doi: 10.2337/diabetes.54.8.2404.
37. Corbin K L, Waters C D, Shaffer B K, et al. Islet Hypersensitivity to Glucose Is Associated With Disrupted Oscillations and Increased Impact of Proinflammatory Cytokines in Islets From Diabetes-Prone Male Mice. *Endocrinology.* 2016;157(5): 1826–1838. doi: 10.1210/en.2015-1879.
38. Muhanhali D, Zhai T, Cai Z, et al. Lipoprotein(a) concentration is associated with risk of type 2 diabetes and cardiovascular events in a Chinese population with very high cardiovascular risk. *Endocrine.* 2020;69(1): 63–72. doi: 10.1007/s12020-020-02286-5.
39. Qi Q, Workalemahu T, Zhang C, et al. Genetic variants, plasma lipoprotein(a) levels, and risk of cardiovascular morbidity and mortality among two prospective cohorts of type 2 diabetes. *Eur Heart J.* 2012;33(3): 325–334. doi: 10.1093/eurheartj/ehr350.
40. Paige E, Masconi K L, Tsimikas S, et al. Lipoprotein(a) and incident type-2 diabetes: results from the prospective Bruneck study and a meta-analysis of published literature. *Cardiovasc Diabetol.* 2017;16(1): 38–44. doi: 10.1186/s12933-017-0520-z.
41. Blue E K, Sheehan B M, Nuss Z V, et al. Epigenetic Regulation of Placenta-Specific 8 Contributes to Altered Function of Endothelial Colony-Forming Cells Exposed to Intrauterine Gestational Diabetes Mellitus. *Diabetes.* 2015;64(7): 2664–2675. doi: 10.2337/db14-1709.

Figures

Figure 1

Comparison of distribution and methylation levels of all differentially methylated genes across the human genome between five GDM samples and five healthy controls. Up-regulated and down-regulated genes after screening are indicated in green and yellow, respectively. The outer ring with numbers 1 to 22 respectively represents 22 autosomes. The inner ring with red and blue pattern shows the methylation levels of DMGs. Circle graph of DMGs throughout autosomal chromosome showing $-\log_{10} (p\text{-Value})$.

Figure 2

(A) Proportion of differentially methylated CpGs identified by RRBS analysis from five GDM samples and five healthy controls in relation to genomic location across the human genome. UTR: untranslated region, CDS: coding domain sequence. (B) The volcano plot of all differentially expressed genes comparing 4 GDM samples with 4 control groups ($p\text{-Value} < 0.05$) including up-regulated genes (yellow dots) and down-regulated genes (green dots) by RNA-Seq analysis.

Figure 3

The Venn graph integrated RRBS and RNA-Seq results showing the whole number of aberrantly methylated-differentially expressed genes overlapped including five up-regulated genes (A) and nineteen down-regulated genes (B). (C) A total of 24 screened genes were shown in heatmap according to the expression levels between four GDM samples and four healthy controls, which were also signed in Fig.1 identifying their location in chromosomes.

Figure 4

Comparison with the methylation level of *TRIM67* CpG sites between 24 GDM samples and 24 healthy controls (NC) by pyrosequencing analysis. Pearson Chi-square test, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Function Classification(GO)

Figure 5

The Gene Ontology (GO) enrichment bar graph of up-regulated genes (red bars) and down-regulated genes (green bars) among all methylated-differentially expressed genes.

Figure 6

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways bubble chart of all methylated-differentially expressed genes. Data are presented as enriched scores expressed as $-\log_{10}(p\text{-Value})$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarytables.docx](#)
- [Supplementaryfig1.png](#)