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Original Research

Down-Regulation of PLAC1 in the Placenta of Gestational Diabetes Mellitus Patients and its Clinical Significance

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Abstract

Background: Placenta-specific 1 (PLAC1) is specifically expressed in the placenta and plays a fundamental role in placenta function. Aberrant expression of PLAC1 has been reported in pregnancy-related disorders; however, its expression in gestational diabetes mellitus (GDM) has not been clearly elucidated. This study aimed to investigate the expression of PLAC1 in the placenta of GDM patients, and its relationship with clinical characteristics. **Methods**: This was a case-control study. Placental tissues were collected from 37 GDM patients (GDM group) and 38 pregnant women with normal glucose tolerance (control group), matched with respect to maternal age and gestational weeks. We examined the expression of PLAC1 in the placenta of both groups and determined its association with clinical indicators. The localization of PLAC1 was confirmed by immunohistochemistry analyses. **Results**: PLAC1 expression was significantly lower in the placenta of GDM patients. For the control group, PLAC1 was positively correlated with pre-pregnancy body mass index (BMI), BMI at delivery, the fasting insulin, triglyceride levels, and homeostasis model assessment during delivery. In the case of GDM patients, there was no correlation between PLAC1 and these indices. Additionally, PLAC1 protein was mainly expressed in the cytoplasm of syncytiotrophoblasts and chorionic stromal cells. **Conclusions**: The expression of PLAC1 was reduced in the GDM placenta, which provides insight into the pathophysiological changes occurring in the placenta of these patients.

Keywords: PLAC1; gestational diabetes mellitus; placenta

1. Introduction

Gestational diabetes mellitus (GDM) corresponds to the first appearance of glucose intolerance during pregnancy [1]. The prevalence of this disorder has been increasing worldwide and was recently reported to be as high as 17.6–24.24% in China [2]. This is of particular concern, given that GDM not only increases the risk of adverse pregnancy outcomes but also has deleterious long-term effects on the health of the mother and offspring [3,4]. The pathogenesis of GDM has not been fully elucidated, although extensive research has suggested that GDM manifests as maternal insulin resistance, inflammation, and placental dysfunction [5].

The placenta, as the key organ for fetal growth and development, plays a vital role in adapting to the maternal environment. Alteration of placental morphology and function impact the intrauterine environment and fetal development [6]. It has been demonstrated that GDM also presents as an enlarged placenta, accompanied by a series of histological changes [7,8]. However, the potential molecular alteration is poorly understood. Placenta-specific 1 (PLAC1) is highly expressed in the placenta, but not in other adult somatic tissues, and plays a fundamental role in placental function and development [9,10]. PLAC1 ablation can lead to placentomegaly and fetal intrauterine growth restriction [10,11]. Previous research has shown lower PLAC1 expression in preeclampsia, which could affect placenta function [12]. Although no studies have shown that PLAC1 is directly related to the onset and/or development of GDM, preeclampsia is one of the complications of GDM, and the placenta is associated with hypoxic changes in both conditions [13,14]. So we speculate that PLAC1 may play a role in the occurrence and development of GDM. Therefore, the objective of this study was to investigate if PLAC1 expression is altered in GDM patients, and whether this change is associated with maternal metabolism and fetal growth.

2. Materials and Methods

2.1 Study Design

Pregnant women who underwent a scheduled cesarean delivery at the obstetric department of Women's Hospital School of Medicine Zhejiang University during July–December 2015 were screened for enrollment. Ethical approval was provided by the hospital board of ethics (ID: 20150045), and informed consent was obtained from all participants. Written informed consent were collected from all subjects prior to peripheral blood and placenta collection. The study included 37 pregnant women with GDM and 38 pregnant women with normal glucose tolerance (control group). GDM was diagnosed based on the

Table 1. Clinical and Laboratory characteristics of the GDM and control groups.

Characteristics	Control (n = 38)	GDM (n = 37)	*p*-value
Maternal age (years)	31.82 ± 3.91	32.81 ± 3.79	0.27
Gestational age (days)	271.39 ± 3.72	271.86 ± 4.26	0.85
Gravidity	2.87 ± 1.36	2.65 ± 1.44	
Parity	0.76 ± 0.49	0.65 ± 0.54	
Pre-pregnancy BMI (kg/m^2)	21.27 ± 2.53	22.56 ± 3.38	0.06
Gestational weight gain (kg)	15.04 ± 3.92	12.94 ± 5.67	0.07
OGTT – FBG (mmol/L)	4.48 ± 0.26	5.29 ± 1.11	**<0.001***,[b]
OGTT – 1 h (mmol/L)	8.19 ± 1.17	10.84 ± 2.58	**<0.001***,[b]
OGTT – 2 h (mmol/L)	6.62 ± 0.91	8.89 ± 1.72	**<0.001***,[b]
BMI at delivery (kg/m^2)	26.99 ± 2.67	27.57 ± 3.75	0.44
FBG (mmol/L)	3.58 ± 0.65	3.98 ± 0.98	**0.04***
Fins (μIU/mL)	7.85 ± 3.59	7.46 ± 3.72	0.66
HOMA-IR	1.27 ± 0.59	1.33 ± 0.88	0.76
TC (mmol/L)	6.67 ± 1.01	6.47 ± 1.17	0.43
TG (mmol/L)	3.61 ± 1.00	4.18 ± 1.83	0.09
HDL (mmol/L)	1.81 ± 0.35	1.70 ± 0.32	0.14
LDL (mmol/L)	3.10 ± 0.69	2.77 ± 0.58	0.07
HbA1c (%)	5.08 ± 0.41	5.44 ± 0.60	**0.02***,[b]
Birthweight (g)	3404.47 ± 337.52	3606.76 ± 445.06	**0.03***,[b]

All values are expressed as mean ± standard deviation. A p-value < 0.05 was taken to indicate a significant difference.

[b]the p-value remaind significant after Least Significance Difference adjustment for multiple comparisons.

p-value in bold indicate significant differences. *, $p < 0.05$.

GDM, gestational diabetes mellitus; BMI, body mass index; OGTT-FBG, oral glucose tolerance test-fasting blood glucose; OGTT, oral glucose tolerance test; FBG, fasting blood glucose; Fins, fasting insulin; HOMA-IR, the homeostatic model assessment of insulin resistance; TC, serum total cholesterol; TG, triglycerides; HDL, high density lipoprotein;; LDL, low density lipoprotein; HbA1c, glycosylated hemoglobin type A1c.

guidelines of the International Association of Diabetes and Pregnancy Study Groups [15], and the control group was matched by maternal age (±2 years) and gestational weeks (±3 days). Pregnant women in the GDM group were given lifestyle-management to control their blood glucose levels during pregnancy. Two pregnant women in the GDM group were treated with insulin to control unsatisfactory blood glucose levels as their 2 hours postprandial blood glucose levels still higher than 6.7 mmol/L after lifestyle-management. The indication for cesarean delivery in both groups included breech presentation, previous cesarean section and macrosomia. Exclusion criteria included twin or multiple pregnancies, congenital malformation of the fetus, and pregnant women with cardiovascular disease, hypertension, liver and kidney diseases, thyroid disease, infection, or diabetes diagnosed before pregnancy.

2.2 Blood Sample and Placental Tissue Collection

The fasting maternal blood were collected prior cesarean section. Serum was isolated from blood samples by centrifugation. Placental tissues were obtained within 10 min after delivery. The tissues from the fetal and maternal surfaces were dissected into small pieces, rinsed with pre-cooled phosphate-buffered saline (PBS) and snap frozen in liquid nitrogen. In addition, placental tissues (1.5–2.5 cm^3) were excised from fetal and maternal sides and fixed with 10% neutral buffered formalin. The serum and frozen tissue were conserved at –80 °C for further analysis.

2.3 Laboratory Assays

Fasting blood glucose (FBG) was measured using an Architect c16000 automated analyzer (Abbott Laboratories, Chicago, IL, USA). Serum total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), fasting insulin (Fins) levels were determined using an Olympus AU400e chemistry immune analyzer (Olympus, Tokyo, Japan). The homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated as follows: HOMA-IR = [fasting glucose (mmol/L) × fasting insulin (μIU/mL)]/22.5.

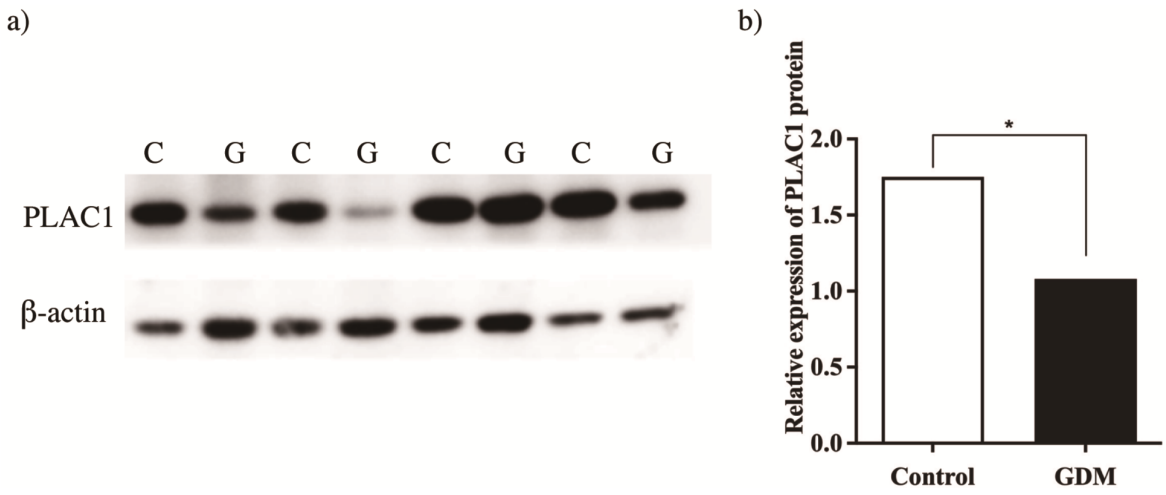

Fig. 1. Expression of PLAC1 protein in placenta from normal glucose tolerance pregnant women (C) and GDM (G) patients. PLAC1 protein extracts from placental tissues of control and GDM patients was determined by Western blotting (a), and the expression level of PLAC1 protein in the GDM group was lower than that that in the control group (b). Value represent mean ± standard deviation. PLAC1, placenta-specific 1; GDM, gestational diabetes.

2.4 Western Blot Analysis

Total protein was extracted from placental tissues by RIPA buffer (Solarbio, Beijing, China) containing protease inhibitor cocktail (Selleckchem, Houston, TX, USA). Protein concentration was measured by the BCA protein assay kit (Thermofisher, Waltham, MA, USA). An equal amount of protein (30 ug) was load in each lane. The protein extract was resolved on SDS-PAGE and transferred to a PVDF membrane (Millipore, Billerica, MA, USA). Membranes were blocked in 5% bovine serum albumin then incubated with the primary antibodies (PLAC1 antibody: ab105395; Abcam, Cambridge, UK; β-actin, Huabio, Hangzhou, Zhejiang, China) and secondary antibodies (Anti-mouse IgG antibody: 7076; Cell Signaling Technology, Boston, MA, USA; Anti-rabbit IgG antibody: 7074; Cell Signaling Technology, Boston, MA, USA). The membranes were treated with an electro-chemiluminescence reagent (Fude Biotech, Guangzhou, Guangdong, China) and visualized using the ImageQuant LAS 4000 mini system (GE Healthcare, Chicago, IL, USA). The gray value of the strip was evaluated using ImageJ software (NIH, Bethesda, MD, USA).

2.5 Immunohistochemistry

The placental specimens were immobilized in 10% neutral buffered formalin and then embedded in paraffin. Slides cut from paraffin blocks were de-paraffinized, rehydrated and incubated with H_2O_2 at 3% for 25 min to block the endogenous peroxidase activity. Antigen retrieval was performed in boiling citrate buffer at pH 6, following by blocking. The same sections were incubated with PLAC1 antibody (PLAC1 antibody: ab117528; Abcam, Cambridge, UK) diluted at 1:50 at 4 °C for overnight and counterstained with horseradish peroxidase (HRP)-conjugated secondary antibodies (Gt anti-Rb-HRP: K5007; DAKO, Copenhagen, Denmark). The final color was developed with 3,3′-diaminobenzidine (Dako/Denmark A/S, Copenhagen, Denmark) and counterstained with Mayer's haematoxylin. The samples were analyzed with an optical microscope by two expert pathologists in double blind.

2.6 Statistical Analysis

Statistical analysis was performed using SPSS 22.0 software (IBM Corp, Chicago, IL, USA). All of the metrological data were normally distributed and are expressed as the mean ± standard deviation ($\bar{x} \pm S$). Comparisons between two groups were conducted using an independent sample t-test. The relative gray value of the placental PLAC1 protein, as measured by Western blot, and other clinical data were subjected to Pearson's correlation analysis. A p-value < 0.05 was considered statistically significant.

3. Results

The characteristics of the study population are shown in Table 1. There were no significant differences in maternal age, gravidity, parity, BMI before pregnancy or at delivery. Compared with normal pregnant women, patients with GDM had higher FBG (3.98 ± 0.98 vs. 3.58 ± 0.65, p = 0.04) and Glycosylated Hemoglobin Type A1c (HbA1c) (5.44 ± 0.60 vs. 5.08 ± 0.41, p = 0.02) at delivery, but fins and HOMA-IR did not significantly differ between the two groups. The birthweight of the GDM group was significantly higher than that of the control group (3606.76 ± 445.06 vs. 3404.47 ± 337.52, p = 0.03). Levels of TC, TG, (high density lipoprotein) HDL and (low density lipoprotein) LDL showed no significant difference between the groups. The expression of PLAC1 in the placenta of

Table 2. Pearson correlations between PLAC1 expression and clinical parameters in the control group.

	r	*p*
Maternal age	–0.15	0.39
Pre-pregnancy BMI	**0.46**	<**0.01****
Gestational weight gain	–0.01	0.96
OGTT – FBG	–0.42	0.80
OGTT – 1 h	0.02	0.92
OGTT – 2 h	–0.06	0.71
BMI at delivery	**0.45**	<**0.01****
FBG	–0.09	0.54
Fins	**0.48**	**0.01***
TC	–0.13	0.42
TG	**0.40**	**0.01***
HDL	–0.24	0.15
LDL	–0.19	0.26
HOMA-IR	**0.43**	**0.01***
HbA1c	–0.22	0.18
Birthweight	0.09	0.61

p-value in bold indicate significant differences. *, $p < 0.05$, **, $p < 0.01$.

PLAC1, placenta-specific 1; r, correlation coefficient; *p*, *p*-value; BMI, body mass index; OGTT-FBG, oral glucose tolerance test-fasting blood glucose; OGTT, oral glucose tolerance test; FBG, fasting blood glucose; Fins, fasting insulin; HOMA-IR, the homeostatic model assessment of insulin resistance; TC, serum total cholesterol; TG, triglycerides; HDL, high density lipoprotein;; LDL, low density lipoprotein; HbA1c, glycosylated hemoglobin type A1c.

Table 3. Pearson correlations between PLAC1 expression and clinical parameters in the GDM group.

	r	*p*
Maternal age	0.05	0.77
Pre-pregnancy BMI	0.31	0.07
Gestational weight gain	–0.11	0.53
OGTT – FBG	0.14	0.41
OGTT – 1 h	0.09	0.60
OGTT – 2 h	0.02	0.91
BMI at delivery	0.22	0.19
FBG	–0.14	0.41
Fins	0.15	0.38
TC	0.10	0.55
TG	0.23	0.17
HDL	0.19	0.25
LDL	–0.17	0.32
HOMA-IR	0.03	0.85
HbA1c	–0.20	0.28
Birthweight	–0.01	0.99

PLAC1, placenta-specific 1; r, correlation coefficient; *p*, *p*-value; GDM, gestational diabetes mellitus; BMI, body mass index; OGTT-FBG, oral glucose tolerance test-fasting blood glucose; OGTT, oral glucose tolerance test; FBG, fasting blood glucose; Fins, fasting insulin; HOMA-IR, the homeostatic model assessment of insulin resistance; TC, serum total cholesterol; TG, triglycerides; HDL, high density lipoprotein;; LDL, low density lipoprotein; HbA1c, glycosylated hemoglobin type A1c.

the control and GDM groups was detected by Western blot analyses. The PLAC1 expression level in the placenta of the GDM group was significantly lower than that of the control group (1.08 ± 0.64 vs. 1.75 ± 1.28, $p < 0.01$) (Fig. 1).

To investigate the relationships of PLAC1 and clinical parameters, we performed Pearson's correlation analyses. In the control group, the expression of PLAC1 protein was significantly correlated with BMI (*r* (correlation coefficient) = 0.45, $p = 0.04$), Fins ($r = 0.48$, $p = 0.01$), TG ($r = 0.40$, $p = 0.01$), and HOMA-IR ($r = 0.43$, $p = 0.01$) (Table 2). In the GDM group, there was no correlation between the relative gray value of the PLAC1 protein and any clinical parameters ($p > 0.05$), as shown in Table 3.

PLAC1 expression and localization was also analyzed by immunohistochemistry. We studied 8 samples of GDM group and 8 samples of control group. Compared to the control group, the placenta of pregnant women in the GDM group (both fetal and maternal surfaces) showed more immature villi. The number of trophoblastic cells, stenosis of the vascular lumen, number of syncytial cells, and number of fibrinoids and necrotic villi were higher in the GDM placentas. PLAC1 protein was mainly expressed in the cytoplasm of the chorionic stroma and syncytiotrophoblasts. PLAC1 protein expression levels in the fetal and maternal surfaces of the placenta were significantly lower in the GDM than control group (Fig. 2).

4. Discussion

Our study demonstrated for the first time that the PLAC1 protein was mainly expressed in the cytoplasm of syncytiotrophoblasts and chorionic cells in the placenta, and was significantly less abundant in the placenta of GDM patients. In the control group of patients with a normal pregnancy, PLAC1 expression was positively correlated with BMI, Fins, TG and HOMA-IR during delivery. In the case of GDM patients, there was no correlation between PLAC1 and these indices, indicating that PLAC1 is closely related to the regulation of metabolic activity *in vivo*, and that the occurrence and development of GDM is associated with a decrease in PLAC1 protein content in the placenta.

Few studies have reported on the expression change of PLAC1 in pregnancy-related disorders. There have been reports of reduced expression of PLAC1 in the placenta, but increased mRNA expression of PLAC1 in the circulation of pre-eclampsia patients, which may be due to the apoptosis of placental chorionic villus cells [16,17]. In addition, decreased PLAC1 expression has been reported in the placenta of patients with fetal growth restriction [18]. Farina

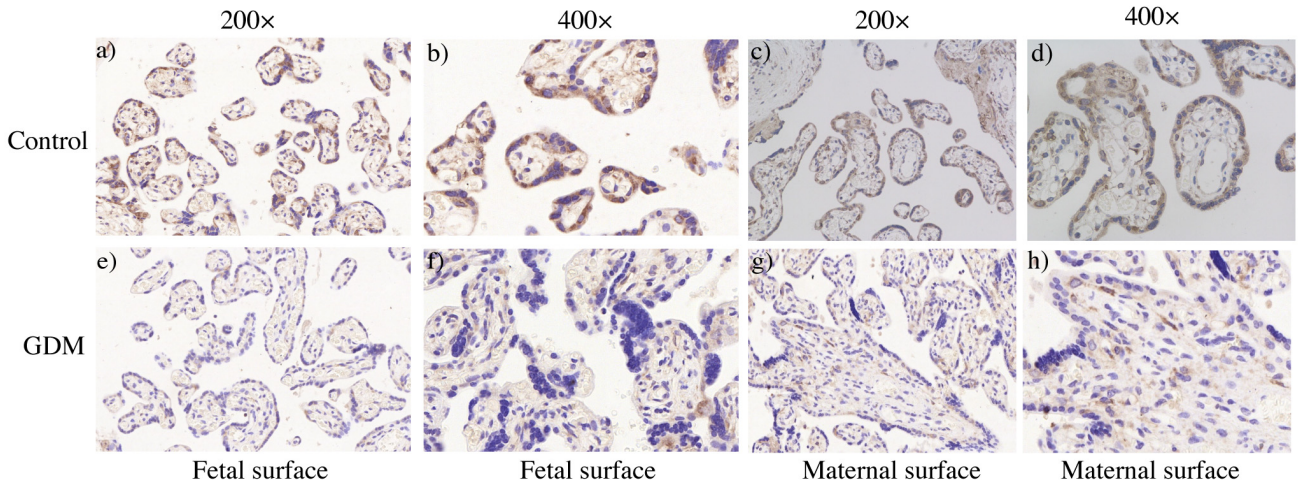

Fig. 2. Expression of PLAC1 in the fetal and maternal surfaces of the control (a–d) and GDM group (e–h) detected by immunohistochemistry. The expression of PLAC1 in the GDM group was lower than that of the control group, and the PLAC1 was located mainly in the cytoplasm of villous stroma and syncytiotrophoblasts. PLAC1, placenta-specific 1; GDM, gestational diabetes.

et al. [19] found that the mRNA level of PLAC1 was lower in the peripheral blood of patients at risk for miscarriage, suggesting that PLAC1 plays a role in regulating the fetal-maternal interface at the early stage of pregnancy. Another study has shown that the persistence of PLAC1 is associated with recurrent pregnancy loss and repeated implantation failure *in vitro* fertilization. The findings of these studies imply that PLAC1 plays a vital role in placental function.

PLAC1 protein is only detected in human placenta; it is not expressed in the decidua or amniotic fluid. Considering that PLAC1 has a highly conserved signal peptide sequence and transmembrane region, we speculated that it may be located in the cell membrane as a receptor, or in the membrane along with membrane organelles, and might participate in cell metabolism and movement, among other functions. Fant *et al*. [20] reported PLAC1 bands in fragments of the endoplasmic reticulum, Golgi apparatus and other organelles, and in plasma membranes, as revealed by Western blot analysis. The immunohistochemical results in this study showed that the brown-stained PLAC1 protein was mainly expressed in the cytoplasm of syncytiotrophoblasts and interstitial cells of villi, in accordance with the above inference.

Previous studies have reported that PLAC1 is aberrantly activated in multiple types of cancer, and is associated with cancer progression [21]. An *in vitro* study showed that a hypoxic environment suppresses the expression of PLAC1 in trophoblast cells [22]. The silencing of PLAC1 expression inhibits the proliferation, migration, and invasion of trophoblasts [12,23]. Chang *et al*. [24] found that down-regulation of PLAC1 gene expression attenuated the syncytialization of cytotrophoblast cells, suggesting that PLAC1 facilitates trophoblast syncytialization. Valent *et al*. [25] demonstrated significantly reduced expression of syncytialization markers in GDM trophoblasts. Thus, we speculate that the abnormal expression of PLAC1 associated with trophoblast syncytialization affects placental function in GDM.

The current study is the first to report abnormal expression of PLAC1 in the GDM placenta. There were several limitations to this study. First, the GDM patients studied had received medical nutritional or insulin therapy before delivery. We only tested FPG and Fins to assess glucose metabolism, which do not necessarily reflect the extent of disease. Second, the sample size was limited. Although the study had the power to detect the differences reported, a larger sample is necessary to confirm our results and validate their clinical relevance. As mentioned above, we speculate that PLAC1 may be involved in the metabolic activities of trophoblast cells and the growth and development of the placenta, cause it is not a secreted protein, So it may be related to some metabolic pathway or the expression of cytokines such as inflammatory cytokines [26]. Those problems require further research to explore.

5. Conclusions

The expression of PLAC1 was reduced significantly in the placentas of GDM patients, as confirmed by Western blotting and immunohistochemical analyses. The results of this study provide insight into the pathophysiological changes that occur in the placentas of GDM patients.

Author Contributions

DC and MDo designed the research study. Initials MDu performed the research. ZL provided help and advice on the experiments. YC analyzed the data. MDu and YC wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Ethical approval was provided by the Women's Hospital School of Medicine Zhejiang University board of ethics (ID: 20150045), and informed consent was obtained from all participants.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2020. Diabetes Care. 2020; 43: S14–S31.

[2] Juan J, Yang H. Prevalence, Prevention, and Lifestyle Intervention of Gestational Diabetes Mellitus in China. International Journal of Environmental Research and Public Health. 2020; 17: 9517.

[3] Lowe Jr WL, Scholtens DM, Kuang A, Linder B, Lawrence JM, Lebenthal Y, *et al*. Hyperglycemia and Adverse Pregnancy Outcome Follow-up Study (HAPO FUS): Maternal Gestational Diabetes Mellitus and Childhood Glucose Metabolism. Diabetes Care. 2019; 42: 372–380.

[4] Saravanan P, Magee LA, Banerjee A, Coleman MA, Von Dadelszen P, Denison F, *et al*. Gestational diabetes: opportunities for improving maternal and child health. The Lancet Diabetes & Endocrinology. 2020; 8: 793–800.

[5] Olmos-Ortiz A, Flores-Espinosa P, Diaz L, Velazquez P, Ramirez-Isarraraz C, Zaga-Clavellina V. Immunoendocrine Dysregulation during Gestational Diabetes Mellitus: The Central Role of the Placenta. International Journal of Molecular Sciences. 2021; 22: 8087.

[6] Ganguly E, Hula N, Spaans F, Cooke CM, Davidge ST. Placenta-targeted treatment strategies: an opportunity to impact fetal development and improve offspring health later in life. Pharmacological Research. 2020; 157: 104836.

[7] Carrasco-Wong I, Moller A, Giachini FR, Lima VV, Toledo F, Stojanova J, *et al*. Placental structure in gestational diabetes mellitus. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2020; 1866: 165535.

[8] Sirico A, Rossi ED, Degennaro VA, Arena V, Rizzi A, Tartaglione L, *et al*. Placental diabesity: placental VEGF and CD31 expression according to pregestational BMI and gestational weight gain in women with gestational diabetes. Archives of Gynecology and Obstetrics. 2022. (in press)

[9] Rawn SM, Cross JC. The Evolution, Regulation, and Function of Placenta-Specific Genes. Annual Review of Cell and Developmental Biology. 2008; 24: 159–181.

[10] Jackman SM, Kong X, Fant ME. Plac1 (placenta-specific 1) is essential for normal placental and embryonic development. Molecular Reproduction and Development. 2012; 79: 564–572.

[11] Fant ME, Fuentes J, Kong X, Jackman S. The nexus of prematurity, birth defects, and intrauterine growth restriction: a role for plac1-regulated pathways. Frontiers in Pediatrics. 2014; 2: 8.

[12] Wan L, Sun D, Xie J, Du M, Wang P, Wang M, *et al*. Declined placental PLAC1 expression is involved in preeclampsia. Medicine. 2019; 98: e17676.

[13] Scifres CM, Parks WT, Feghali M, Caritis SN, Catov JM. Placental maternal vascular malperfusion and adverse pregnancy outcomes in gestational diabetes mellitus. Placenta. 2017; 49: 10–15.

[14] Li HP, Chen X, Li MQ. Gestational diabetes induces chronic hypoxia stress and excessive inflammatory response in murine placenta. International Journal of Clinical and Experimental Pathology. 2013; 6: 650–659.

[15] Metzger BE, Gabbe SG, Persson B, Lowe LP, Dyer AR, Oats JJN, *et al*. International Association of Diabetes and Pregnancy Study Groups Recommendations on the Diagnosis and Classification of Hyperglycemia in Pregnancy. Diabetes Care. 2010; 33: 676–682.

[16] Zanello M, Sekizawa A, Purwosunu Y, Curti A, Farina A. Circulating mRNA for the PLAC1 Gene as a second Trimester Marker (14-18 Weeks' Gestation) in the Screening for Late Preeclampsia. Fetal Diagnosis and Therapy. 2014; 36: 196–201.

[17] Kodama M, Miyoshi H, Fujito N, Samura O, Kudo Y. Plasma mRNA concentrations of placenta-specific 1 (PLAC1) and pregnancy associated plasma protein a (PAPP-a) are higher in early-onset than late-onset pre-eclampsia. Journal of Obstetrics and Gynaecology Research. 2011; 37: 313–318.

[18] Sun D, Wu H, Ping Z, Zhu H, Ai L. PLAC1 Regulates the Occurrence of Fetal Growth Restriction by Inhibiting the Apoptosis of Trophoblast Cells. Annals of Clinical and Laboratory Science. 2021; 51: 182–189.

[19] Yilmaz N, Timur H, Ugurlu EN, Yilmaz S, Ozgu-Erdinc AS, Erkilinc S, *et al*. Placenta specific protein-1 in recurrent pregnancy loss and in *In Vitro* Fertilisation failure: a prospective observational case-control study. Journal of Obstetrics and Gynaecology. 2020; 40: 843–848.

[20] Fant M, Barerra-Saldana H, Dubinsky W, Poindexter B, Bick R. The PLAC1 protein localizes to membranous compartments in the apical region of the syncytiotrophoblast. Molecular Reproduction and Development. 2007; 74: 922–929.

[21] Mahmoudian J, Ghods R, Nazari M, Jeddi-Tehrani M, Ghahremani MH, Ghaffari-Tabrizi-Wizsy N, *et al*. PLAC1: biology and potential application in cancer immunotherapy. Cancer Immunology, Immunotherapy. 2019; 68: 1039–1058.

[22] Devor EJ, Santillan DA, Warrier A, Scroggins SM, Santillan MK. Placenta-specific protein 1 (PLAC1) expression is significantly down-regulated in preeclampsia via a hypoxia-mediated mechanism. The Journal of Maternal-Fetal & Neonatal Medicine. 2021; 1–7.

[23] Chang W, Yang Q, Zhang H, Lin H, Zhou Z, Lu X, *et al*. Role of placenta-specific protein 1 in trophoblast invasion and migration. Reproduction. 2014; 148: 343–352.

[24] Chang W, Wang H, Cui L, Peng N, Fan X, Xue L, *et al*. PLAC1 is involved in human trophoblast syncytialization. Reproductive Biology. 2016; 16: 218–224.

[25] Valent AM, Choi H, Kolahi KS, Thornburg KL. Hyperglycemia and gestational diabetes suppress placental glycolysis and mitochondrial function and alter lipid processing. The FASEB Journal. 2021; 35: e21423.

[26] Sirico A, Dell'Aquila M, Tartaglione L, Moresi S, Farì G, Pitocco D, *et al*. PTH-rP and PTH-R1 Expression in Placentas from Pregnancies Complicated by Gestational Diabetes: New Insights into the Pathophysiology of Hyperglycemia in Pregnancy. Diagnostics. 2021; 11: 1356.