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Original Article

Correlation between methylation levels of TRIM28 gene at Cpg10-11 sites in patients with gestational diabetes mellitus and infant obesity

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Abstract: Objective: We aimed to investigate the correlation between methylation levels of TRIM28 gene at CpG10-11 sites in patients with gestational diabetes mellitus (GDM) and infant obesity. Methods: A total of 513 GDM patients were selected for the study. Meanwhile, 213 healthy pregnant women with normal glucose tolerance (NGT) were assigned to a control group. After delivery, 5 mL cord blood was collected from each subject for detecting the methylation degree of TRIM28 gene at CpG10-11 sites using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) method. A multivariate regression model was applied to analyze the correlation between the methylation level of TRIM28 gene at CpG10-11 sites and infant obesity at birth and 6, 12, and 18 months old. Results: The methylation level of TRIM28 gene at CpG3 site was positively correlated with the birth weight ($P<0.05$). The methylation levels of TRIM28 gene at CpG sites had no correlation with the infant weight at 6 months, weight gain during the 6 months, weight-for-age BMI Z-Score, and weight-for-age Z-Score (all $P>0.05$). After the confounding factors were adjusted, the mean methylation levels of TRIM28 gene at CpG sites showed a positive correlation with the infant weight at 12 months, weight gain from 6 to 12 months, and weight-for-age Z-Score (all $P<0.05$). Conclusion: The methylation level of TRIM28 gene at CpG10-11 sites and the mean methylation levels of TRIM28 gene at CpG sites were significantly correlated with BMI and age-specific BMI Z-Score of infants, and the correlation was most significant at 18 months.

Keywords: Gestational diabetes mellitus, tripartite motif-containing 28, CpG10-11

Introduction

With the development of society and the improvement in living standards, the problem of infant obesity has been taken seriously, and the traditional view is that the increased occurrence of obesity may be related to the improved nutrition level, decreased physical activities, and genetic factors [1]. Further studies have shown that the intrauterine high-glucose environment in patients with gestational diabetes mellitus (GDM) may also be a significant cause of infant obesity [2]. It is currently believed that the biological mechanism of GDM affecting infant obesity may be related to hyperinsulinemia and epigenetic changes, and the latter is the dominant factor [3]. Epigenetics believes that without changing the gene sequence, different environment can result in different epigenetic modifications of genes and then may cause different biological effects [4]. DNA methylation is the most common change of epigenetic modification [5, 6]. As a 'switch' in the gene for obesity, TRIM28 (Tripartite motif protein 28) was found to play a crucial role in 'fetal programming'. At the same time, experiments in animals found that the distribution of body weights of mice with TRIM28 mutation was bimodal, and the incidence rate of obesity was significantly increased [7, 8]. Based on the above research findings, this study speculates that the high-glucose environment in GDM patients can lead to the initiation of 'fetal obesity programming', which then causes the changes in the methylation levels of TRIM28 gene and ultimately results in obesity.

Materials and methods

Study subjects

A total of 513 GDM patients treated in Tengzhou Central People's Hospital between January 2014 and December 2015 for labor were selected and assigned to a GDM group (age: 28.6±4.3 years, pre-pregnancy BMI: 23.6±4.2 kg/m^2). Meanwhile, 213 healthy pregnant women with normal glucose tolerance (NGT) were assigned to a control group (NGT group). This research has been approved by the Ethics Committee of Tengzhou Central People's Hospital.

Inclusion and exclusion criteria

Inclusion criteria: 1) patient who was 18 to 42 years old; 2) patient who had singleton pregnancy confirmed by color ultrasound; 3) patient who was 37 to 42 weeks pregnant; 4) patient who had received at least senior high school education; 5) clinical data was complete; 6) patient who was healthy without other diseases; 7) patient who consented to be involved in the study.

Exclusion criteria: 1) patient who was under 18 years of age or above 42 years of age; 2) patient who had twin pregnancy or multiple pregnancies; 3) patient who gave birth prematurely; 4) patient who had not received senior high school education; 5) patient who had incomplete clinical data; 6) patient who suffered from any of the chronic diseases including pregestational diabetes mellitus, gestational hypertension, preeclampsia, hyperthyroidism, hypothyroidism, and severe renal impairment; 7) patient who refused to be involved in the study.

Diagnostic criteria for GDM

The diagnostic criteria used in the study was based on the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria for GDM, according to which the diagnosis was made if any of the following abnormal values appeared after an oral glucose tolerance test (OGTT): 1) fasting plasma glucose level ≥5.1 mmol/L; 2) one-hour plasma glucose level ≥10 mmol/L; 3) two-hour plasma glucose level ≥8.5 mmol/L.

Evaluation methods for markers of obesity

The birth weight and length of 20,000 babies born in Tengzhou Central People's Hospital in the past ten years were used as criteria. The newborns who had birth weight greater than the 90th percentile for their gestational age were considered large for gestational age (LGA); newborns who had birth weight below the 10th percentile were considered small for gestation age (SGA); and newborns who had birth weights between the two percentiles were considered appropriate for gestational age (AGA). Obesity-related indicators were calculated using the Z-Score formula: Z-Score = (measured value (of height or weight) - mean of standard (height or weight))/standard deviation of standard (height or weight). Specific evaluation indicators included gestational age-specific birth weight Z-Score, age-specific BMI Z-Score, and weight-for-age Z-Score.

Detection of the methylation level of TRIM28 gene

On the premise of obtaining informed consent from all the study objects, 5 mL cord blood was collected after delivery, centrifuged at 1,000 rpm for 10 minutes, numbered, and then stored in a refrigerator at -80°C. After completing all the collection, guanidine hydrochloride method was used for DNA extraction, and the information of TRIM28 gene and promoter region CpG islands was obtained using NCBI and UCSC databases. MassArray, a methylation-specific primer design software, was used to design primers, and T7-promoter sequence was added to the 5' end of the reverse primer and 10 mer tag to the 5' end of the forward primer. Specific primer sequences and CpG sites are shown in **Tables 1** and **2**. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) was employed for detection. The mass spectrum peak was detected using EpiTYPER software, and the degree of methylation was assessed based on the mass spectrum peak figure.

PCR reaction system

The total volume was 5 μL, which included 1.42 μL double distilled water, 0.5 μL PCR buffer (10×), 0.04 μL dNTP and enzyme, 1 μL forward and reverse primers, and 1 μL DNA samples to be measured. The reaction conditions are shown in **Figure 1**.

Statistical analysis

All data were analyzed using SPSS Statistics 21.0. Measurement data are represented by

Table 1. PCR amplification primers of TRIM28 gene

	Sequence	Number of CpG Island	Segment
10F	aggaagagagTTTTTTTTATTTGTGGTTAGGTTGG	11	487
T7R	cagtaatacgACTCATAGGGGGAGAAGGCAATAATTCCCCCT		

Table 2. Position of each CpG site of TRIM28 gene

CpG site	Chromosome	Position of CpG site
CpG1	chr19	59055377
CpG2		59055389
CpG3		59055414
CpG4		59055412
CpG5		59055458
CpG6		59553698
CpG7		59555874
CpG8		59558952
CpG9		59557458
CpG10		59555963
CpG11		59553308

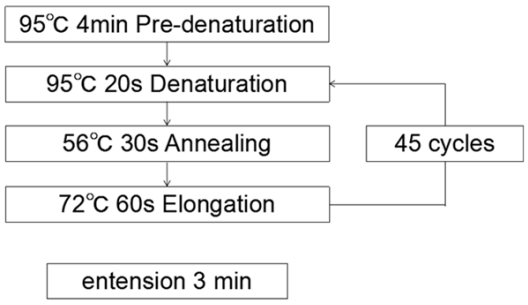

Figure 1. PCR reaction conditions.

mean ± sd, and count data are expressed as n, %. When the methylation level of TRIM28 gene did not conform to the normal distribution, the median was used as a reference value. The patients whose level was no less than the reference value composed a reference group. Single-variant logistic analysis was used to analyze the correlation between the various factors, with α=0.05 as the significance level.

Results

Basic information of the subjects

The GDM group and the NGT group were comparable in terms of maternal age, pre-pregnancy BMI, marital status, and annual household income per capita (P>0.05). There were also no differences in gestational age at delivery, parity, and newborns' gender (P>0.05, **Table 3**).

Comparison of blood glucose levels during pregnancy and birth weight of newborns in the two groups

As shown in **Table 4**, levels of the first trimester glycosylated hemoglobin and second-trimester OGTT markers including fasting blood glucose, glucose concentration at one-hour glucose load, and glucose concentration at two-hour glucose load of the patients in the GDM group were significantly higher than those in the NGT group (P<0.001). The fasting blood glucose levels in the two groups during the first trimester were similar (P>0.05). The birth weight, gestational age-specific birth weight Z-score, and the proportion of LGA and fetal macrosomia in the GDM group were significantly higher than those in the NGT group (P<0.01).

Correlation between methylation levels of TRIM28 gene and markers related to birth weight

Statistical analysis showed that the methylation level of TRIM28 gene at CpG3 site was positively correlated with birth weight, and the relations between methylation levels at other sites and the birth weight as well as gestational age-specific birth weight Z-Score were not statistically significant. See **Table 5**.

Correlation between methylation level of TRIM28 gene and markers related to growth and development in 6-month old infants

At 6th month of the study, 13 babies were lost to follow-up, so information of 500 infants was collected in this phase. Statistical analysis showed that there were no correlations between the methylation level of TRIM28 gene at CpG sites and the weight of 6-month old infants, weight gain during the 6 months, BMI, weight-for-age Z-Score, and age-specific BMI Z-Score. See **Table 6**.

Correlation between methylation level s of TRIM28 gene and markers related to growth and development in 12-month-old infants

At 12th month of the study, more babies were lost to follow-up. Information of only 460 infants

Table 3. Basic information of the subjects

Variable	GDM group (n=513)	NGT group (n=213)	*P* value
Maternal age	25.3±3.65	26.8±1.96	0.695
Pre-pregnancy BMI	21.6±2.36	20.9±2.02	0.952
Marital status (yes/no)	502/11	208/5	0.654
Annual household income per capita (ten thousand)	3.2±0.36	3.3±0.58	0.512
Gestational age at delivery	39.5±0.99	39.4±1.02	0.485
Parity (1/2)	480/33	208/5	0.852
Newborn's gender (male/female)	265/248	108/105	0.721

Note: GDM, gestational diabetes mellitus; NGT, normal glucose tolerance; BMI: body mass index.

Table 4. Comparison of blood glucose level during pregnancy and birth weight of newborns in the two groups

Variable	GDM group (n=513)	NGT group (n=213)	P
First trimester			
FBG (mmol/L)	5.01±0.63	4.96±0.33	0.254
HbA1c (%)	5.4±0.51	5.3±0.44	0.066
Second trimester (OGTT)			
0 h	5.3±0.21	4.2±0.31	0.000
1 h	8.9±1.25	6.9±2.01	0.000
2 h	7.4±1.36	6.5±1.02	0.000
Birth weight (kg)	3.9±0.45	3.1±0.66	0.000
Gestational age-specific birth weight Z-Score	0.5±0.91	-0.5±1.03	0.000
Birth weight (based on gestational age)			
SGA	13 (2.53)	16 (7.51)	0.000
AGA	361 (70.37)	180 (84.51)	
LGA	139 (27.10)	17 (7.98)	
Birth weight			
Normal	410 (79.92)	203 (95.31)	0.000
Fetal macrosomia	103 (20.08)	10 (4.69)	

Note: GDM, gestational diabetes mellitus; NGT, normal glucose tolerance; LGA: large for gestational age; SGA: for gestation age; AGA: appropriate for gestational age; FBG, fasting blood glucose; HbA1c: hemoglobin A1c; OGTT: oral glucose tolerance test.

was collected in this phase. After the confounding factors were adjusted in the statistical analysis, the mean methylation level of TRIM28 gene at each CpG site (CpGM#) showed a positive correlation with the infant weight at 12 months, weight gain from 6-12 months, and weight-for-age Z-Score. See **Table 7**.

Correlation between methylation levels of TRIM28 gene and markers related to growth and development in 18-month old children

In this phase, clinical data of 400 children were collected. The analysis showed that the methylation levels of TRIM28 gene at CpG 10-11 and CpGM sites were positively correlated with the markers related to growth and development in 18-month-old children. The results are shown in **Table 8**.

Discussion

The World Health Report 2004 stated that about 10% of children were overweight or obese at that time, and the obesity rate of the children aged 0 to 6 in China was roughly equal to that number [9-10]. Related studies have indicated that childhood obesity not only impairs children's health in childhood but also may cause various life-threatening diseases in adulthood. Therefore, active control of children's weight can have positive impact. For GDM patients, the probability of giving birth to large babies or overweight babies is greatly increased. The problem of infant obesity seriously affects the health of offspring, which required a focused clinical study [11-14].

At present, the relationship between GDM and obesity in patients' offspring is still unclear. Epigenetics provides a possible explanation for one of the molecular mechanisms of obesity in GDM patients' offspring. It is believed that necessary mutations can occur in some genes according to the environment, so to some extent, these kinds of mutations can reflect the changes in the environment where the off-

Table 5. Correlation between methylation level of TRIM28 gene and birth weight

Variable	Birth weight (kg)		Gestational age-specific birth weight Z-Score	
	β (SE)	P	β (SE)	P
CpGM#	33.63 (23.56)	0.361	0.08 (0.06)	0.315
CpG1-2	-19.36 (16.32)	0.254	-0.05 (0.04)	0.541
CpG3	31.28 (15.23)	0.049	0.07 (0.04)	0.041
CpG4	-3.58 (20.21)	0.845	-0.02 (0.03)	0.666
CpG5-6-7	15.36 (14.02)	0.327	0.01 (0.06)	0.841
CpG8-9	12.36 (13.02)	0.125	0.02 (0.03)	0.329
CpG10-11	9.36 (11.02)	0.587	0.03 (0.04)	0.116

Note: #represents the mean methylation level of TRIM gene at CpG sites; SE: Standard error.

Table 6. Correlation between methylation levels of TRIM28 gene and markers related to growth and development in 6-month-old infants

Variable	Weight (kg)		Weight gain in 0-6 months (kg)		BMI (kg/m^2)		Weight-for-age Z-Score		Age-specific BMI Z-Score	
	β (SE)	P	β (SE)	P	β (SE)	P	β (SE)	P	β (SE)	P
CpGM#	0.04 (0.03)	0.563	0.04 (0.03)	0.205	0.04 (0.04)	0.763	0.06 (0.05)	0.554	0.03 (0.04)	0.212
CpG1-2	0.03 (0.05)	0.196	0.05 (0.04)	0.151	0.03 (0.06)	0.851	0.04 (0.03)	0.354	0.04 (0.03)	0.071
CpG3	0.01 (0.04)	0.543	0.05 (0.03)	0.062	0.01 (0.05)	0.663	0.05 (0.03)	0.606	0.04 (0.06)	0.176
CpG4	0.05 (0.05)	0526	0.02 (0.01)	0.611	-0.05 (0.06)	0574	-0.05 (0.04)	0.611	-0.02 (0.01)	0.601
CpG5-6-7	0.01 (0.04)	0.336	0.01 (0.02)	0.141	0.01 (0.04)	0.136	0.06 (0.04)	0.081	0.05 (0.04)	0.343
CpG8-9	0.03 (0.05)	0.522	0.02 (0.03)	0.332	-0.03 (0.01)	0.510	-0.02 (0.03)	0.112	0.07 (0.02)	0.804
CpG10-11	0.05 (0.04)	0.517	0.03 (0.04)	0.075	0.05 (0.02)	0.219	0.03 (0.04)	0.854	-0.03 (0.01)	0.375

Note: #represents the mean methylation level of TRIM gene at CpG sites; BMI: body mass index; SE: Standard error.

Table 7. Correlation between methylation levels of TRIM28 gene and markers related to growth and development in 12-month old infants

Variable	Weight (kg)		Weight gain in 6-12 months (kg)		Weight-for-age Z-Score	
	β (SE)	P	β (SE)	P	β (SE)	P
CpGM#	0.14 (0.08)	0.033	0.09 (0.16)	0.041	0.11 (0.13)	0.025
CpG1-2	0.02 (0.04)	0.106	0.05 (0.03)	0.211	0.03 (0.02)	0.101
CpG3	0.03 (0.03)	0.053	0.04 (0.02)	0.762	0.03 (0.03)	0.162
CpG4	-0.04 (0.02)	0.516	-0.02 (0.02)	0.601	-0.02 (0.012)	0.677
CpG5-6-7	0.11 (0.04)	0.056	0.10 (0.08)	0.101	0.06 (0.04)	0.103
CpG8-9	0.12 (0.04)	0.512	0.13 (0.23)	0.072	0.11 (0.10)	0.302
CpG10-11	-0.05 (0.04)	0.507	-0.03 (0.04)	0.145	-0.03 (0.04)	0.175

Note: #represents the mean methylation level of TRIM gene at CpG sites; SE: Standard error.

spring stay in. As the blood glucose level of the GDM patients is high, their babies often take too much glucose in. Under this kind of internal environment, gene mutations occur in babies, and then a large amount of sugar gets converted into fat, resulting in obesity of the offspring [15-17]. During the fetal development process, the fetuses are very sensitive to the intrauterine environment. In order to adapt to the high glucose level in the environment, they will stay or change their high glucose state. The fetuses undergo epigenetic modifications during the development process, leading to the over conversion of sugar to fat. The epigenetic modifications that occur under these conditions may be permanent and have a long-term impact on the health of the offspring. They will not disappear after the babies are born. Even the babies stay away from the high-sugar environment, their sensitivity to sugar and the conversion efficiency from sugar to fat remain unchanged. As a "switch" gene for obesity, TRIM28 is turned on in a high-sugar environment, and then the obesity process is started. However, the "on" position will not be changed after the birth of the babies. It will not be turned off and still works even

Table 8. Correlation between methylation levels of TRIM28 gene and markers related to growth and development in 18-month old children

Variable	BMI (kg/m^2)		Age-specific BMI Z-Score	
	β (SE)	P	β (SE)	P
CpGM#	0.14 (0.09)	0.043	0.17 (0.11)	0.045
CpG1-2	0.06 (0.05)	0.096	0.08 (0.05)	0.101
CpG3	0.06 (0.03)	0.503	0.05 (0.04)	0.072
CpG4	0.08 (0.11)	0.510	0.07 (0.05)	0.211
CpG5-6-7	-0.05 (0.03)	0.096	0.06 (0.07)	0.131
CpG8-9	0.04 (0.05)	0.574	0.02 (0.03)	0.232
CpG10-11	0.15 (0.12)	0.037	0.18 (0.09)	0.049

Note: #represents the mean methylation level of TRIM gene at CpG sites; BMI: body mass index; SE: Standard error.

though the babies are no longer stay in the high-sugar intrauterine environment. Therefore, TRIM28 works as a promoter to obesity in the "fetal programming" process [18-20]. This study found that the methylation level of TRIM28 gene at CpG3 site was positively correlated with the birth weight. Although negative results were obtained at 6 months, the methylation levels of TRIM28 gene at CpG10-11 sites and CpGM levels were positively correlated with the BMI and age-specific BMI Z-Score of the children at 18 months. These findings suggest that the increase in methylation levels of TRIM28 gene results in the decrease in the expression levels of TRIM28 gene in vivo, which leads to an increase in BMI and age-specific BMI Z-Score in 18-month-old children. In another similar study by He et al., it was found that in addition to birth weight, feeding patterns, time of complementary feeding, and genetic factors, especially TRIM28 genes, can influence infant obesity as well [21].

In conclusion, the study results showed that GDM may raise the BMI and age-specific BMI Z-Score of infants by increasing the methylation levels of TRIM28 gene at CpG10-11 sites and the CpGM levels. As time went on, this influence was strengthened at 18 months. In this study, the methylation levels at the CpG10-11 sites were found to be associated with BMI and age-specific BMI Z-Score of the babies, however, the mechanism of how methylation at the CpG10-11 sites induces the increase in the levels of BMI and age-specific BMI Z-Score of the babies were not explained and its molecular biological mechanism remained unclear. Therefore, further study needs to be conducted. Moreover, we cannot draw a conclusion whether the decrease in methylation levels at CpG10-11 sites can reduce BMI and age-specific BMI Z-Score of the babies from this study, thus this will be a focus in our future studies.

Disclosure of conflict of interest

None.

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References

[1] You W and Henneberg M. Cereal crops are not created equal: wheat consumption associated with obesity prevalence globally and regionally. Aims Public Health 2016; 3: 313-328.

[2] Holmes AV, Auinger P and Howard CR. Combination feeding of breast milk and formula: evidence for shorter breast-feeding duration from the national health and nutrition examination survey. J Pediatr 2011; 159: 186-191.

[3] Gimenez-Marin A and Rivas-Ruiz F. Clinical governance and patient safety culture in clinical laboratories in the spanish national health system. Rev Calid Asist 2017; 32: 303-315.

[4] Quevedo SF, Bovbjerg ML and Kington RL. Translation of fetal abdominal circumference-guided therapy of gestational diabetes complicated by maternal obesity to a clinical outpatient setting. J Matern Fetal Neonatal Med 2017; 30: 1450-1455.

[5] Lee H, Park H, Ha E, Hong YC, Ha M, Park H, Kim BN, Lee B, Lee SJ, Lee KY, Kim JH, Jeong KS and Kim Y. Effect of breastfeeding duration on cognitive development in infants: 3-year follow-up study. J Korean Med Sci 2016; 31: 579-584.

[6] Bhang SY, Ha E, Park H, Ha M, Hong YC, Kim BN, Lee SJ, Lee KY, Kim JH, Jeong J, Jeong KS, Lee B and Kim Y. Maternal stress and depressive symptoms and infant development at six months: the mothers and children's environmental health (MOCEH) prospective study. J Korean Med Sci 2016; 31: 843-851.

[7] Boucher O, Julvez J, Guxens M, Arranz E, Ibarluzea J, Sanchez de Miguel M, Fernandez-Somoano A, Tardon A, Rebagliato M, Garcia-Esteban R, O'Connor G, Ballester F and Sunyer J.

Association between breastfeeding duration and cognitive development, autistic traits and ADHD symptoms: a multicenter study in Spain. Pediatr Res 2017; 81: 434-442.

[8] Llewellyn A, Simmonds M, Owen CG and Woolacott N. Childhood obesity as a predictor of morbidity in adulthood: a systematic review and meta-analysis. Obes Rev 2016; 17: 56-67.

[9] Di Bernardo S, Mivelaz Y, Epure AM, Vial Y, Simeoni U, Bovet P, Estoppey Younes S, Chiolero A and Sekarski N. Assessing the consequences of gestational diabetes mellitus on offspring's cardiovascular health: mysweetheart cohort study protocol, Switzerland. BMJ Open 2017; 7: e016972.

[10] Cote S, Gagne-Ouellet V, Guay SP, Allard C, Houde AA, Perron P, Baillargeon JP, Gaudet D, Guerin R, Brisson D, Hivert MF and Bouchard L. PPARGC1alpha gene DNA methylation variations in human placenta mediate the link between maternal hyperglycemia and leptin levels in newborns. Clin Epigenetics 2016; 8: 72.

[11] Er-Di XU, Yin CY, Chang M and Xiao Y. Investigation and analysis of childhood obesity-related factors. Chinese Journal of Child Health Care 2013.

[12] Gill SV, Maybenson TA, Teasdale A. Birth and developmental correlates of birth weight in a sample of children with potential sensory processing disorder. BMC Pediatrics 2013; 13: 29-29.

[13] Young LE. Imprinting of genes and the Barker hypothesis. Twin Res 2001; 4: 307-317.

[14] Dalgaard K, Landgraf K, Heyne S, Lempradl A, Longinotto J, Gossens K, Ruf M, Orthofer M, Strogantsev R, Selvaraj M, Lu TT, Casas E, Teperino R, Surani MA, Zvetkova I, Rimmington D, Tung YC, Lam B, Larder R, Yeo GS, O'Rahilly S, Vavouri T, Whitelaw E, Penninger JM, Jenuwein T, Cheung CL, Ferguson-Smith AC, Coll AP, Korner A and Pospisilik JA. Trim28 haploinsufficiency triggers bi-stable epigenetic obesity. Cell 2016; 164: 353-364.

[15] Zhao YL, Ma RM, Lao TT, Chen Z, Du MY, Liang K, Huang YK, Zhang L, Yang MH, Sun YH, Li H and Ding ZB. Maternal gestational diabetes mellitus and overweight and obesity in offspring: a study in Chinese children. J Dev Orig Health Dis 2015; 6: 479-484.

[16] Wang H, Jiang H, Yang L and Zhang M. Impacts of dietary fat changes on pregnant women with gestational diabetes mellitus: a randomized controlled study. Asia Pac J Clin Nutr 2015; 24: 58-64.

[17] Wang C, Zhu W, Wei Y, Feng H, Su R and Yang H. Exercise intervention during pregnancy can be used to manage weight gain and improve pregnancy outcomes in women with gestational diabetes mellitus. BMC Pregnancy Childbirth 2015; 15: 255.

[18] Klimczak M, Czerwinska P, Mazurek S, Sozanska B, Biecek P, Mackiewicz A and Wiznerowicz M. TRIM28 epigenetic corepressor is indispensable for stable induced pluripotent stem cell formation. Stem Cell Res 2017; 23: 163-172.

[19] Huang Y, Li Z, Fang F, Qin Z, Yan S and Wang YF. Long-term effects of gestational diabetes mellitus on mothers and offspring and strategies of postpartum follow up. Journal of Shanghai Jiaotong University 2016.

[20] Xiong X, Saunders LD, Wang FL. Gestational diabetes mellitus: prevalence, risk factors, maternal and infant outcomes. Int J Gynaecol Obstet 2001; 75: 221-228.

[21] Xing-Yan HE, Su-Mei OU, Chen W and Chen LZ. Correlation analysis of multiple factors for the risk factors of nurseling obesity. China Modern Medicine 2015.