Effect of *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val polymorphisms on susceptiblity to gestational diabetes mellitus

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ABSTRACT. We investigate the role of the *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val genetic polymorphisms in the susceptibility to gestational diabetes mellitus. A total of 223 pregnant women with gestational diabetes mellitus and 265 healthy pregnant women were examined at The Second Affiliated Hospital of Shaanxi University of Chinese Medicine from May 2013 to November 2013. Genotyping for detection of *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val polymorphisms was conducted using the restriction fragment length polymorphism-polymerase chain reaction. There were statistically significant differences between patients with gestational diabetes mellitus and control subjects in terms of age ($\chi^2 = 6.68$, P = 0.01) and BMI ($t = 7.56$,

P < 0.001) levels of HDL-C (t = 2.62, P = 0.005) and LDL-C (t = 3.98, P < 0.001). By the chi-square test, we found significant differences between the present and null genotype distributions of *GSTM1* (χ^2 = 10.95, P = 0.0009). Null genotype of *GSTM1* could influence the susceptibility to gestational diabetes mellitus compared to the present genotype [adjusted OR (95%CI) = 1.85 (1.26-2.72)]. However, the unconditional logistic analysis revealed that *GSTT1* and *GSTP1* IIe105Val polymorphisms could not influence the risk of gestational diabetes mellitus in a Chinese population. In summary, we suggest that the *GSTM1* gene polymorphism could influence the susceptibility to gestational diabetes mellitus in a Chinese population.

Key words: *GSTM1*; *GSTT1*; *GSTP1* IIe105Val; Polymorphism; Gestational diabetes mellitus

INTRODUCTION

Gestational diabetes mellitus is defined as carbohydrate intolerance during pregnancy (Metzger et al., 2007). The etiology of gestational diabetes mellitus is not well-understood. The pathogenesis of gestational diabetes mellitus involves many complex environmental and lifestyle factors, such as pregnancies at an older age, high pre-pregnancy weight and BMI, hydramnios, a history of gestational diabetes mellitus, a history of type 2 diabetes, vaginal candida infection, and a history of gestational diabetes (Kühl, 1991; Damm, 1998; Kopp, 2005; Anna et al., 2008; Savitz et al., 2008; Sella et al., 2013; Moon et al., 2015). However, not all pregnant women exposed to certain risk factors go on to develop gestational diabetes mellitus, suggesting that hereditary factors may contribute to the occurrence of this disease. Currently, many epidemiologic studies have shown that certain genes, such as melatonin receptor type 1B (MTNR1B), lipoprotein lipase gene, potassium channel voltage gated KQT-like subfamily Q, member 1 (KCNQ1), methylenetetrahydrofolate reductase (MTHFR), factor V Leiden (FVL), and adiponectin gene and adenosine deaminase, contribute to the susceptibility to gestational diabetes mellitus (Khan et al., 2014; Ao et al., 2015; Li et al., 2015a; Takhshid et al., 2015a,b).

Glutathione-S-transferase (GST) is a family of cytosolic enzymes that have a critical role in catalyzing the conjugation of glutathione with a broad spectrum of endogenous and exogenous compounds, detoxifying environmental toxins and preventing reactive oxygen species-mediated cell injury in the body (Strange et al., 1998; Hayes et al., 2005). Three functional genes have been found in the GST family: GST Mu 1 *(GSTM1*), GST Theta 1 (*GSTT1*), and *GST Pi 1* (GSTP1). The gene variations of GSTs can alter the expression of enzyme and affect their detoxification ability. In the present study, we investigate the role of the *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val genetic polymorphisms in the susceptibility to gestational diabetes mellitus.

MATERIAL AND METHODS

Patients

A total of 223 pregnant women with gestational diabetes mellitus were examined at The Second Affiliated Hospital of Shaanxi University of Chinese Medicine from May 2013

to November 2013. The pregnant women with gestational diabetes mellitus aged 20-40 years old. The gestational diabetes mellitus was diagnosed according to the criteria established by the American Diabetes Association (2008), with gestational age being between 24 and 28 weeks and the overnight glucose tolerance test being 75 g at 2 h after an overnight fast. The exclusion criteria for patients were pre-existing diabetes, abnormal result in a glucose screening test prior to gestational age being 24th week, multiple gestations, liver or kidney disease, or endocrine disorders.

A total of 265 pregnant women without gestational diabetes mellitus were randomly recruited from pregnant women who received regular prenatal care in The Second Affiliated Hospital of Shaanxi University of Chinese Medicine from May 2013 to November 2013. The exclusion criteria were control subjects who had a history of hypertension, diabetes, multiple gestations, liver or kidney disease, or endocrine disorders.

The biochemical data were collected by face-to-face interview from a self-designed questionnaire. The clinical data were collected from medical records. The demographic information included gender, age, body mass index (BMI), tobacco use (smoking), alcohol consumption, and history of gestational diabetes mellitus. The clinical information included total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). All patients and subjects provided written informed consent. the Second Affiliated Hospital of Shaanxi University of Chinese Medicine approved the performance of this study.

DNA extraction and genotyping

Peripheral venous blood was drawn from patients and control subjects in this study. DNA was extracted from the blood samples using the Qiagen blood mini kit (Qiagen, Hilden, Germany) following the manufacturer's recommendation. Genotyping for detection of *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val polymorphisms was conducted using the restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) method. The forward and reverse primers for *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val polymorphisms were designed using the Sequenom Assay Design 3.1 software (San Diego, CA, USA). The PCR conditions were as follows: started at 95°C for 5 min for initial denaturation; followed by 35 cycles of amplification with denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s; and a final extension step of 72°C for 5 min for all the polymorphic sites. The resulted fragments were electrophoresized on 2% agarose gel stained with ethidium bromide to determine the genotypes of the subjects for all the polymorphic sites. To confirm the genotyping results, 10% PCR-amplified DNA samples were examined by DNA sequence, and the results were 100% concordant.

Statistical methods

The statistical variation within the demographic variables of the two study groups was done using the chi-square test for the categorical data and the Student *t*-test for continuous variables. Conformation to Hardy-Weinberg equilibrium was tested using the chi-square test or the Fisher exact test. To evaluate the association between *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val polymorphisms and gestational diabetes mellitus risk, the unconditional logistic regression analysis was used to calculate adjusted odds ratio (OR) along with their 95%

confidence intervals (CIs). The ORs and 95%CI were adjusted for potential confounding factors. Statistical analysis was conducted using the SPSS 17.0 package (SPSS Inc., Chicago, IL, USA). $P < 0.05$ indicated statistical significance.

RESULTS

The mean ages of patients were 31.52 ± 9.25 years, and were 28.21 ± 9.83 years for controls (Table 1). There were statistically significant differences between patients with gestational diabetes mellitus and control subjects in terms of age ($\chi^2 = 6.68$, $P = 0.01$) and BMI ($t = 7.56$, $P < 0.001$) levels of HDL-C ($t = 2.62$, $P = 0.005$) and LDL-C ($t = 3.98$, $P < 0.001$). However, no significant differences were found in gestational age ($t = 0.39$, $P = 0.35$), smoking ($\chi^2 = 2.18$, $P = 0.14$), alcohol consumption ($\chi^2 = 0.06$, $P = 0.81$), or levels of TC ($t = 1.42$, $P = 0.08$) or TG ($t = 1.57$, $P = 0.06$).

The chi-square test revealed that the genotype frequencies of *GSTP1* IIe105Val did not deviate from the Hardy-Weinberg equilibrium in the control group, and the P value for Hardy-Weinberg equilibrium was 0.51 (Table 2). There was significant difference in genotype distributions in terms of *GSTM1* ($\chi^2 = 10.95$, $P = 0.0009$). However, No significant differences were observed in the genotype distributions with respect to *GSTT1* ($\chi^2 = 0.99$, $P = 0.32$) and *GSTP1* IIe105Val ($\chi^2 = 1.78$, $P = 0.41$). Null genotype of *GSTM1* could influence the susceptibility to gestational diabetes mellitus compared to the present genotype, and the adjusted OR (95%CI) was 1.85 (1.26-2.72). However, the unconditional logistic analysis revealed that *GSTT1* and *GSTP1* IIe105Val polymorphisms could not influence the risk of gestational diabetes mellitus in a Chinese population.

Table 1. Characteristics of patients with gestational diabetes mellitus and control subjects.

Variables	Patients (N = 223)	%	Controls (N = 265)	%	Chi-square test or Student *t*-test	P value
Age (years)	31.52 ± 9.25		28.21 ± 9.83		3.81	<0.001
<30	106	47.53	157	59.25		
≥30	117	52.47	108	40.75	6.68	0.01
Gestational age	27.71 ± 4.87		27.53 ± 5.12		0.39	0.35
BMI (kg/m^2)	30.72 ± 5.64		27.15 ± 4.80		7.56	<0.001
<28	68	30.49	166	62.64		
≥28	155	69.51	99	37.36	50.15	<0.001
Smoking						
No	215	96.41	261	98.49		
Yes	8	3.59	4	1.51	2.18	0.14
Alcohol consumption						
No	211	94.62	252	95.09		
Yes	12	5.38	13	4.91	0.06	0.81
TG (mg/dL)	255.20 ± 108.52		269.81 ± 117.33		1.42	0.08
TC (mg/dL)	232.73 ± 46.42		239.30 ± 45.70		1.57	0.06
HDL-C (mg/dL)	50.61 ± 12.50		53.46 ± 11.48		2.62	0.005
LDL-C (mg/dL)	113.22 ± 24.79		122.52 ± 26.43		3.98	<0.001

BMI, body mass index; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

The gene-environmental analysis was performed to evaluate the role of age, BMI, and levels of HDL-C and LDL-C in the association between the *GSTM1* polymorphism and the risk of gestational diabetes mellitus (Table 3). However, this gene polymorphism indicated no association with age, BMI, or levels of HDL-C and LDL-C in influencing the susceptibility to gestational diabetes mellitus.

Table 2. Association between *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val polymorphisms and development of gestational diabetes mellitus.

Genotypes	Patients (N = 223)	%	Controls (N = 265)	%	Chi-square test	P value	OR (95%CI)[1]	P value
GSTM1								
Present	117	52.47	178	67.17			1.0 (Reference)	-
Null	106	47.53	87	32.83	10.95	0.0009	1.85 (1.26-2.72)	<0.001
GSTT1								
Present	148	66.37	187	70.57			1.0 (Reference)	-
Null	75	33.63	78	29.43	0.99	0.32	1.21 (0.81-1.81)	0.32
GSTP1 IIe105Val								
Codominant								
IIe/IIe	95	42.60	128	48.30			1.0 (Reference)	-
IIe/Val	99	44.39	109	41.13			1.22 (0.82-1.82)	0.30
Val/Val	29	13.00	28	10.57	1.78	0.41	1.40 (0.75-2.61)	0.26
Dominant								
IIe/IIe+IIe/Val	194	87.00	237	89.43			1.0 (Reference)	-
Val/Val	29	13.00	28	10.57	0.70	0.40	1.27 (0.70-2.29)	0.40
Recessive								
IIe/IIe	95	42.60	128	48.30			1.0 (Reference)	-
IIe/Val+Val/Val	128	57.40	137	51.70	1.59	0.21	1.26 (0.87-1.83)	0.21

[1]Adjusted for age, BMI, HDL-C and LDL-C.

Table 3. Relationship between the GSTM1 polymorphism and demographic and clinical characteristics in the risk of gestational diabetes mellitus.

Variables	Patients (N = 223)		Controls (N = 265)		OR (95%CI)	P value
	Present (117)	Null (106)	Present (178)	Null (87)		
Age (years)						
<30	54	52	105	52	1.94 (1.14-3.33)	0.009
≥30	63	54	73	35	1.79 (1.00-3.19)	0.03
BMI (kg/m^2)						
<28	34	34	110	56	1.96 (1.06-3.63)	0.02
≥28	83	72	68	31	1.90 (1.09-3.36)	0.02
HDL-C (mg/dL)						
<50	56	50	75	37	1.81 (1.01-3.25)	0.03
≥50	61	56	103	50	1.89 (1.12-3.20)	0.01
LDL-C (mg/dL)						
<120	73	66	82	40	1.85 (1.09-3.17)	0.02
≥120	44	40	96	47	1.86 (1.03-3.35)	0.03

DISCUSSION

Gestational diabetes mellitus is a common disease in pregnant women worldwide, and the pathogenesis of this disease is still unclear. In recent years, the important role of genetic predisposition played in lung cancer has promoted substantial interest. Single nucleotide polymorphisms refer to the alteration of a single nucleotide base, by insertion, deletion, or replacement, and thus influence the expression and function of protein (Friedberg, 2003). In this study, we assessed the relationship between *GSTM1*, *GSTT1* and *GSTP1* IIe105Val polymorphisms and gestational diabetes mellitus risk in a Chinese population, and we observed that the *GSTM1* genetic polymorphism plays an important role in risk of gestational diabetes mellitus in the Chinese population.

The genotype distributions for *GSTM1* genetic polymorphisms in control subjects were inconsistent with the results of previous studies in Chinese population (Chiyomaru et al., 2011; Yu et al., 2011; Jiang et al., 2012; Senthilkumar Thirumurugan, 2012; Xu et al., 2013; Li

et al., 2012, 2015b). The frequency of the *GSTM1* null genotype was 32.83%, which is similar to the genotype frequencies observed in Asian population and African American populations (about 30%), but is different from that in Caucasians (about 50%). Previous epidemiologic studies have reported a significant relationship between *GSTM1* genetic polymorphisms and development of several kinds of disorders, such as hypertension, hepatotoxicity, type 2 diabetes, ischemic stroke, and cancers (Wang et al., 2012; Gupta et al., 2013; Liu et al., 2013; Eslami and Sahebkar, 2014; Petrovič and Peterlin, 2014; Weich et al., 2015). These studies have shown that the *GSTM1* genetic polymorphism could influence the susceptibility to human diseases.

For the correlation between *GSTM1* genetic polymorphism and development of gestational diabetes mellitus, only one study investigated their relationship (Orhan et al., 2014). Orhan et al. (2014) carried out a case-control study involving 50 women with GDM and 50 control subjects, and they investigated whether the *GSTM1* and *GSTT1* genetic variations contributed to susceptibility of gestational diabetes mellitus. However, they did not reveal a statistically significant relationship between the two genetic polymorphisms and gestational diabetes mellitus risk. In our study, we revealed that only the *GSTM1* null genotype was correlated with breast cancer risk in a Chinese population, which is inconsistent with previous results. Further studies with large scale sample sizes are greatly needed to verify our results.

In summary, we determined the relationship between *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val genetic variations and development of gestational diabetes mellitus in a Chinese population. We suggest that the *GSTM1* gene polymorphism could influence the susceptibility to gestational diabetes mellitus in a Chinese population.

Conflicts of interest

The authors declare no conflict of interest.

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