RESEARCH ARTICLE

The CDKN2A polymorphisms and the susceptibility of HBV-related gestational diabetes mellitus

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Background: Single-nucleotide polymorphisms (SNPs) were discovered in HBV-related gestational diabetes mellitus, but it still unclear whether these SNPs are associated with the susceptibility of HBV-related gestational diabetes mellitus.

Methods: The investigation of the association between CDKN2A polymorphisms and occurrence of HBV-related gestational diabetes mellitus (GDM) in Chinese was assessed in the case-control study. A total of 480 pregnant patients with HBV and 530 pregnant controls were consecutively recruited from January 2015 to December 2016. Polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) method was applied to measure genotyping for the detection of CDKN2A.

Results: The significant differences in the frequency of CDKN2A genotype distributions, rs10811661 and rs564398, were found by Chi-square test. Using conditional logistic analysis, individuals carrying the CDKN2A rs10811661 TC and TT genotypes and CDKN2A rs564398 AA and AG genotypes were related to a greater risk of HBV-related GDM compared with the genotype.

Conclusions: In conclusion, the CDKN2A rs10811661 and rs564398 polymorphisms showed association with a greater risk of HBV-related GDM in a Chinese population.

KEYWORDS

CDKN2A, HBV-related gestational diabetes mellitus, polymorphism

1 | INTRODUCTION

One of the common diseases occurred in pregnant women is gestational diabetes mellitus (GDM). A report showed that the incidence of GDM was from the range of 2.4%-22.3% among pregnant women in the worldwide.¹ The development of gestational diabetes mellitus could result from complex environmental factors, lifestyle factors, and genetic mutation.

However, not all pregnant women developed GDM even although they exposed to the same risks of GDM, which indicated that the genetic influences might contribute to the development of GDM.² Cumulative evidence suggested that the genetic mutations contributed to the development of GDM, including KCNQ1, MTHFR, FVL, and CDKN2A.³⁻⁶

The cyclin-dependent Kinase Inhibitor 2A (CDKN2A), one member in cyclin-dependent kinase family, is at chromosome 9, band

p21.3 in human. It is reported that several variations of CDKN2A have associated with diabetes. The single-nucleotide polymorphisms (SNPs) rs10811661 and rs564398 in upstream of CDKN2A were reported to be risk factors of diabetes.⁷ Recent studies demonstrated hepatic genetic mutations by maternal hepatitis B virus (HBV) infection (HBsAg positive) could influence the function of liver, leading to hepatic metabolic disorders and even hepatocellular carcinoma (HCC).⁸⁻¹¹ In addition, CDKN2A mutations have close association of hepatic metabolic disorders induced by hepatitis B virus.¹² Thus, there was a suggested association between genetic polymorphisms of CDKN2A and development of HBV-related gestation diabetes mellitus.

Therefore, the case-control study to examine the relation between CDKN2A polymorphisms and occurrence of HBV-related GDM in a Chinese population was conducted.

2 | MATERIALS AND METHODS

2.1 | Patients and ethics statement

The case-control study of 480 pregnant women diagnosed as HBV-positive was consecutively collected from our hospital between January 2015 and December 2016. All pregnant women were between 25 and 40 years old. The diagnostic criteria of HBV were dependent on serologic tests. Gestational age was between 12 and 28th week of gestation. The inclusion criteria were patients with HBV infection confirmed by serologic tests and tested by a 2-hour 75 g OGTT at the first antenatal visit. Patients with pre-existing diabetes and mixed HBV and HCV infection were excluded.

During the same space of time, a total of 530 pregnancy subjects without HBV were randomly selected from regular prenatal examiners in the Department of Obstetrics and Gynecology. These examiners were regarded as controls, who had a history of hypertension, diabetes, HBV, and mixed HBV and HCV infection were excluded.

The self-designed questionnaire or medical examination records were demographic characteristics (Gender, age, Body Mass Index (BMI), and the natural history of HBV) and clinical characteristics of pregnant women with HBV and control individuals. Total cholesterol (TC), triglyceride (TG), low-density lipopolysaccharide cholesterol (LDL-c), high-density lipopolysaccharide (HDL-c), systolic blood pressure (SBP), blood uric acid (BUA), and serum insulin were included in clinical characteristics. The informed consents were signed and then collected from pregnant women with HBV, and control individuals, respectively. The design of this study was approved by the ethics committee of Xuzhou maternity and child healthcare hospital according to the Declaration of Helsinki.

2.2 | GDM risk analysis

Body height, body weight, and sitting blood pressure (SBP) after no less than 10-min rest were analyzed. Due to the little weight gain at the first 12-week gestation, the weight at the registration was considered to be prepregnancy body weight. The difference of body weight from registration to GCT was calculated from the gain of weight from prepregnancy to glucose challenge test (GCT). BMI was the body weight (kilogram) average divided by square of height (meters). The criteria of obesity and overweight were on the basis from the Working Group on Obesity (WGOC) in China, which defined the BMI $\geq 28.0 \text{ kg/m}^2$ as obesity, BMI between 24.0 and 27.9 kg/m^2 as overweight, BMI between 18.5 and 23.9 kg/m^2 as normal weight, and BMI $<18.5 \text{ kg/m}^2$ as underweight.

2.3 | Extraction and genotyping of DNA

Patients and control individuals were drawn 5 mL venous blood at the beginning of the study. The separation of the plasma content from blood specimens was first collected in tubes with anticoagulants and then centrifuged. The EDTA-treated whole blood was used to extract genomic DNA by the DNAzol BD reagent according to manufacturer's protocol (ThermoFisher, USA). PCR-RFLP was applied to detect genotyping

of CDKN2A. The detection of CDKN2A genotyping was amplified through the forward and reverse primers. For CDKN2A, the forward and reverse primers were 5'-GGAGCCATTCTATCGTGAACA-3' and 5'-CACCCGACTGGAAAACCTTAT-3', respectively. The fragment lengths for CDKN2A were 475 bp. The PCR conditions were as following at 95°C for 5 minutes for initial denaturation, 35 cycles of 95°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds for amplification, and finishing at 72°C for 4 minutes. The success rate of genotyping was 100%.

2.4 | Statistical methods

The means $\pm$ standard deviation expressed continuous variables, and N (%) of participants represented categorical variables in the study. The characteristic variances between patients with HBV-related GDM and control individuals were calculated by the student *t* test or chi-square test. The association between CDKN2A and risk of HBV-related GDM was measured by conditional logistic regression analysis. The odds ratio (OR) and 95% confidence intervals (CIs) were also shown in results. The reference group was defined as wide-type genotype of CDKN2A. The statistical analysis was processed by SPSS 17.0 package (SPSS Inc., Chicago, IL, USA).

3 | RESULTS

3.1 | The association between HBV and gestational diabetes mellitus

The case-control study of 480 pregnant women diagnosed as HBV-positive and 530 HBV-negative ones was performed to study the association between HBV and gestational diabetes mellitus. The average ages of patients and controls were 28.29 ± 3.54 and 27.00 ± 3.95 years, respectively. By student *t* test, patients with HBV were more likely to have higher BMI, fasting plasma glucose, and HOMA-IR, level of BUA and TC, when compared with the controls. However, no obvious difference between two groups in the gestational age ($P = 0.33$), SBP ($P = 0.31$) and levels of TG ($P = 2.11$), LDL-c ($P = 0.05$), and HDL-c ($P = 0.13$). The details of these characteristics in HBV patients and controls were shown in Table 1.

3.2 | The association between CDKN2A polymorphisms and gestational diabetes mellitus

Genotype and allele distributions of CDKN2A variants, rs10811661 and rs564398, among the HBV patients and the control individuals were displayed in Table 2. For CDKN2A rs10811661, two genotypes, TT and TC, have differences in the genotype and frequencies in allele distribution between the HBV patients and the controls. The people with the CDKN2A rs564398 AA and AG genotypes have a significantly increased risk of HBV-related GDM in comparison with the GG genotype (Table 2).

The CDKN2A polymorphisms were associated with age, BMI, fasting plasma glucose, and HOMA-IR for the risk of GDM (Table 3 and 4). We found CDKN2A polymorphisms were related to increased risk of GDM in HBV patients regardless of age.

TABLE 1 Characteristics between patients with gestational diabetes mellitus and control subjects

Variables	HBV patients N = 480	%	Controls N = 530	%	Student t test	P value
Age, years	28.29 ± 3.54		27.00 ± 3.95		4.69	0.22
<30	310	64.58	370	69.81		
≥30	170	35.41	160	30.19		
Gestational age	27.82 ± 3.72		26.62 ± 3.97		4.31	0.33
BMI, kg/m ²	26.38 ± 1.84		21.96 ± 3.18		19.63	<0.001
<28	370	77.08	490	92.45		
≥28	110	22.91	40	7.55		
Fasting plasma glucose, mg/dL	114.32 ± 19.73		94.92 ± 10.54		3.71	<0.001
HOMA-IR	5.56 ± 0.75		3.23 ± 0.45		21.16	<0.001
SBP, mm Hg	110.75 ± 11.73		109.29 ± 10.97		3.05	0.31
BUA, umol/L	345.15 ± 34.79		170.49 ± 27.11		220.88	<0.001
Insulin, uIU/mL	74.0 ± 23.12		18.0 ± 5.67		103.84	<0.001
TC, mmol/L	4.42 ± 1.53		2.31 ± 1.82		5.14	<0.001
TG, mmol/L	2.17 ± 1.26		1.32 ± 1.45		0.16	2.11
LDL-C, mmol/L	3.07 ± 1.03		2.98 ± 1.16		0.61	0.05
HDL-C, mmol/L	1.64 ± 0.34		1.58 ± 0.23		0.79	0.13

TABLE 2 Genotype distributions and allele frequencies of CDKN2A polymorphisms in patients and controls and their association with HBV-related gestational diabetes mellitus

SNPs	Genotypes	HBV Patients N = 480	%	Controls N = 530	%	OR (95% CI) ^a	P value ^a
rs10811661							
Dominant model	CC	70	14.58	140	26.42	1	
	TC	210	43.75	200	37.74	0.67 (0.38-1.02)	<0.001
	TT	200	41.67	190	35.85	0.43 (0.19-0.95)	<0.001
Allele	C	350	22.92	480	25.00	1	
	T	610	77.08	580	75.00	0.47 (0.40-0.54)	<0.001
rs564398							
Dominant model	AA	50	10.42	110	58.33	1	
	AG	200	39.58	220	34.86	1.01 (0.75-1.41)	<0.001
	GG	230	50.00	200	5.50	1.18 (0.80-1.70)	<0.001
Allele	A	290	30.21	440	41.51	1	
	G	670	69.79	620	58.49	0.86 (0.70-1.03)	<0.001

^aAdjusted for age, and BMI.

4 | DISCUSSION

The same exposure to environmental and lifestyle factors would not induce the development of the same diseases. It indicated the importance of genetic mutation in contribution to the development of diseases. The variations in a single nucleotide at a specific position in the genome were called as the single-nucleotide polymorphisms (SNPs). The variation is including to insert, miss, or replace a single nucleotide, which induces the polymorphism in the nucleic acid

sequence.¹³ These changes in gene polymorphisms will influence gene expression, and function of their translational products.^{14,15} In the study, a case-control investigation to study whether CDKN2A polymorphisms could influence the susceptibility to GDM in HBV patients was conducted. We found that the CDKN2A mutations were related to a greater risk of GDM.

The CDKN2A locus is on chromosome 9 p21.3. The dysfunction of liver in HBV patients was reported to associate with CDKN2A mutation. In addition, the polymorphisms in CDKN2A could decrease or

TABLE 3 Interaction between CDKN2A rs10811661 polymorphism and demographic and clinical characteristics in the risk of gestational diabetes mellitus

Variables	HBV Patients			Controls			TC vs CC		TT vs CC	
	CC	TC	TT	CC	TC	TT	OR (95% CI) ^a	P value ^a	OR (95% CI) ^a	P value ^a
Age, years										
<30	80	90	100	120	110	140	0.03 (0.01-0.17)	0.657	0.03 (0.01-0.15)	0.507
≥30	50	60	60	60	50	50	0.02 (0.01-0.16)	0.463	0.02 (0.01-0.16)	0.423
BMI, kg/m ²										
<28	50	160	170	170	160	160	0.26 (0.15-0.43)	0.001	0.28 (0.17-0.45)	0.001
≥28	20	40	50	10	10	20	0.16 (0.11-0.53)	<0.001	0.17 (0.13-0.34)	<0.001
Fasting plasma glucose, mg/dL										
<80	0	10	10	10	10	0	N/A	N/A	N/A	N/A
≥80	60	210	200	160	170	170	0.36 (0.24-0.54)	0.003	0.33 (0.22-0.51)	0.002
HOMA-IR										
<4.0	0	0	10	180	170	160	N/A	N/A	0.43 (0.39-0.68)	0.23
≥4.0	70	230	170	10	10	0	0.39 (0.27-0.57)	0.003	N/A	N/A

^aAdjusted for age.**TABLE 4** Interaction between CDKN2A rs564398 polymorphism and demographic and clinical characteristics in the risk of gestational diabetes mellitus

Variables	HBV Patients			Controls			AA vs GG		AG vs GG	
	AA	AG	GG	AA	AG	GG	OR (95% CI) ^a	P value ^a	OR (95% CI) ^a	P value ^a
Age, years										
<30	100	110	90	120	120	130	0.03 (0.01-0.18)	0.301	0.03 (0.01-0.19)	0.261
≥30	50	60	60	60	50	50	0.02 (0.01-0.16)	0.283	0.02 (0.01-0.17)	0.245
BMI, kg/m ²										
<28	70	140	170	140	180	170	0.17 (0.08-0.34)	<0.001	0.24 (0.14-0.42)	<0.001
≥28	10	40	60	10	20	10	0.06 (0.02-0.19)	0.001	0.11 (0.05-0.24)	0.008
Fasting plasma glucose, mg/dL										
<80	0	10	10	0	10	10	N/A	N/A	N/A	N/A
≥80	70	210	190	160	170	170	1.50 (1.11-2.03)	0.001	1.58 (1.12-2.23)	<0.001
HOMA-IR										
<4.0	0	0	10	190	160	160	N/A	N/A	N/A	N/A
≥4.0	80	220	170	10	10	0	1.57 (1.15-2.16)	0.001	N/A	N/A

^aAdjusted for age.

eliminate detoxification of enzyme and increase the susceptibility to diseases, like GDM.

Accumulating studies have shown that CDKN2A polymorphisms presented in liver samples from HCC, chronic hepatitis, and liver cirrhosis patients.¹⁶ The CDKN2A polymorphisms in above diseases were strongly associated with HBV (LIVINGSTON S et al 2007). It was reported that HBV infection disrupted hepatic metabolic responses (SHI YX et al 2016). Thus, CDKN2A mutations suggested association between HBV and hepatic metabolic function. In addition, the association between CDKN2A polymorphisms and risk of GDM in this study indicated that CDKN2A

polymorphisms were associated with the occurrence of GDM.¹⁷ Currently, no report illustrated the relationship between polymorphisms of CDKN2A and risk of HBV-related GDM. This study investigated in a population with 480 GDM and 530 control individuals, and we found that CDKN2A polymorphisms were associated with the risk of HBV-related GDM.

In our study, we found that the CDKN2A mutations were correlated with the development of GDM in HBV patients. However, small sample size in our study was limited to our findings, which was better to further examine in large sample sizes. Moreover, there are still limitations in our study. First, samples were collected from one

hospital, which might not represent the general population. Second, other genetic polymorphisms might contribute to develop gestational diabetes mellitus in HBV patients and CDKN2A gene polymorphisms. Third, the statistical error might occur because of small size sample. In conclusion, we suggest that the CDKN2A mutations are correlated with an increased risk of HBV-related GDM in the Chinese population. This finding was useful for studying the genetic features of HBV patients with GDM and developing more efficient preventive and therapeutic strategies.

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