Effect of placental sex hormone-binding globulin single nucleotide polymorphism rs6259 on protein and function in gestational diabetes mellitus

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Abstract. Sex hormone-binding globulin (SHBG) has a key role in the occurrence and development of the gestational diabetes mellitus (GDM). Single nucleotide polymorphism (SNP) rs6259 is a functional site in SHBG gene, which is suspected to regulate the SHBG level. The present study explored the placental SHBG SNP rs6259 distribution in Chinese pregnant women and the influence on placental SHBG concentrations, to assess the relationship of SHBG rs6259 in the occurrence and development of GDM. We screened the SHBG rs6259 allele in 210 healthy and 180 GDM gravidas by PCR-RFLP and restriction enzyme and measured placental SHBG concentrations in each genotypic group with western blot analysis. The mechanisms of SHBG rs6259 function were analyzed by cell culture, recombinant lentivirus transfection, real-time PCR, and western blot analysis. We found the differences of SHBG Asn^{327} allele frequency and the genotype distribution in GDM and control groups were statistically significant ($P<0.05$). Western blot analysis results showed that the Asn^{327} allele group was associated with a higher placental SHBG level than the Asp^{327} allele homozygote group ($P<0.05$). In HTR8-SVneo cell transfection, the positive transfection groups (SHBG-rs6259 Asn) led to an obviously higher tendency of SHBG mRNA and protein expression than the negative control groups (SHBG-rs6259 Asp), the normal cell group, and the blank control group (blank lentivirus LV-5) ($P<0.05$). Our data, therefore, reflected that SHBG SNP rs6259 causes changes in placental SHBG concentration and may play a functional role in the molecular mechanisms of GDM etiology.

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Introduction

Gestational diabetes mellitus (GDM) is defined as abnormal or impaired glucose metabolism before pregnancy and it initially appears during pregnancy. GDM may lead to early embryo abnormalities or even death, pregnancy-induced hypertension syndrome, infection, polyhydramnios and premature labor. It will also affect the status of the fetus, including effects such as fetal malformation, macrosomia or stillbirth. The offspring of woman with GDM are more inclined to develop low blood sugar, respiratory distress syndrome and polycythemia. Furthermore, these offspring have a higher risk of developing obesity and impaired glucose tolerance and the mother with GDM is more likely to develop diabetes [mainly type 2 diabetes mellitus (DM)] later in life.

In recent years, the incidence of GDM has increased significantly. In China, the incidence of GDM has increased significantly and women with GDM account for 3-7% of pregnancies (1). The pathophysiological basis of GDM world-wide includes increased insulin resistance and decreased secretion of β-cell during pregnancy (2,3). Sex hormone-binding globulin (SHBG) is a glycoprotein produced in the liver, specifically to bind and transport sex hormones, and regulate the biological activity and the concentration of sex hormone in the blood (4). Previous studies have shown that the level of SHBG was decreased in women with insulin tolerance and GDM (5). A low SHBG level is an independent risk factor for type 2 DM, which has been suggested as a predictor for hyperinsulinemia and insulin tolerance (6). Higher SHBG concentrations can effectively prevent impaired fasting glucose, the occurrence of type 2 DM and hyperinsulinemia (7).

Placenta is a combination of tissues of embryo and mother, carrying the exchange of substances. It consists of amniotic membrane, chorion frondosum and deciduas. Placenta is evolved from the fertilized egg and has the same genetic material with the fetus. Our prior experiments on the changes of SHBG in GDM placental tissue have been confirmed that SHGB can be synthesized and secreted by the

placenta trophoblast cells, SHBG mRNA and SHBG protein concentration were significantly decreased in the GDM group (P<0.01), and that of SHBG played an important role in the occurrence and development of GDM (8-10).

The single-nucleotide polymorphism, rs6259, is a functioning gene mutation within a coding region of the SHBG gene. Nucleotide 5790 within exon 8 of the SHBG gene has a missense mutation (GAC→AAC), and the carboxyl-terminal globular laminin domain created a new N-connecting carbohydrate chain, which could provide for an additional N-glycosylation site (11,12). Glycosylation does not affect the binding of steroids to SHBG, but it may decrease the plasma clearance of SHBG and prolong its half-life (13,14). This substitution may lead to increased SHBG levels, changes in gene polymorphism (TAAAA)n and has influence on transcription of the non-coding region (11). Studies have confirmed that the increased SHBG levels in hirsutism (11) and polycystic ovary syndrome (3) are associated with the variant Asn^{327} allele. Ding *et al* (15) found that Asn^{327} allele carriers have higher SHBG levels in plasma (10%) than Asp^{327} carriers (P=0.005). This single nucleotide polymorphism (SNP) rs6259 has different frequencies in ethnic groups around the world (13). In healthy Caucasians it was 7.5-12%, in African Americans 2%, and the highest frequency was found in Chinese women (16-21).

The present study focused on the distribution of the human SHBG SNP rs6259, its correlation with placental SHBG levels and the occurrence and development of GDM, to predict the risk of diabetes in the next generation.

Patients and methods

Patients and sample collection. All subjects were from Shengjing Hospital, which is affiliated with China Medical University. This study was approved by the Ethics Committee of Shengjing Hospital, and all participants provided informed consent.

In total, 180 women with singleton pregnancy, positive oral glucose tolerance test (OGTT), and regular prenatal examinations were selected into the study. The normal group was comprised of 210 cases that were OGTT negative, healthy pregnant women with singleton pregnancy over the same period. Pregnant women with a complication, such as gestational hypertension and pregestational DM were excluded. The GDM group was selected from the outpatients whose glucose was well-controlled and who received systematic examinations during the pregnancy from November 2012 to September 2014. According to the American Diabetes Association (22), the recommended GDM diagnosis is: i) fasting plasma glucose over 5.1 mmol/l during pregnancy; and ii) blood glucose levels at 1 and 2 h after taking 75-g oral glucose over 10.0 and 8.5 mmol/l during gestational weeks 24 to 28. Either one beyond the diagnostic boundary can be identified as GDM.

The placental tissues of the two groups were collected from each patient immediately after delivery. The tissue was cut into ~0.5 cm^3 pieces from placenta on the side of the mother, central and marginal areas. Cold normal saline was used to wash the pieces before they were dissected into small sections, which were blotted dry on filter paper and snap-frozen in liquid nitrogen for 4 h, before being stored at -80˚C.

Clinical index collection. We had statistics on general information in the selected 390 cases, including in pregnant women the age, gestational weeks, birth weight, childbearing history and fasting blood glucose, and the differences were compared between the two groups.

DNA sample preparation. Genomic DNA was extracted from placental samples by a blood/cell/tissue genomic DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing, China) strictly according to the manufacturer's instructions. The test result determination must take the microtiter plate reader as a standard.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and agarose gel electrophoresis. PCR primer sequences were as follows: forward primer, 5'-TTCTGGATCCGAGCCACCT-3' and reverse primer, 5'-AGTGCCTGGTACATTGCTAG-3' (Invitrogen Life Technologies, Carlsbad, CA, USA). Each PCR reaction contained, DNA template 2 μl, Takara Ex Taq (5 U/μl) 0.25 μl, dNTP mixture (2.5 mM) 4 μl, 1 μl (10 μM) each of SHBG forward and reverse primers, 10X Ex Taq buffer (Mg^{2+} Plus) 5 μl, with ultrapure water added to 50 μl total volume (Takara Bio, Inc., Otsu, Japan). The PCR conditions: initial denaturation at 94˚C for 5 min. PCR profiles consisted of 35 cycles with denaturation at 94˚C for 10 sec, annealing at 55˚C for 30 sec, and extension at 72˚C for 60 sec, followed by a final extension at 72˚C for 7 min. PCR products were electrophoresed in a 2% agarose gel and visualized by ethidium bromide and UV light (c150; Azure Biosystems, Inc., Dublin, CA, USA).

Restriction enzyme digestion technique. According to the SNP mutation site gene, we selected the restriction enzyme *Hin*fI (Takara Bio, Inc.) for the treatment of PCR amplified fragments, to carry on the identification of the gene. All of the 180 GDM and 210 in the control group PCR amplified fragments were by *Hin*fI. The reaction system contained Takara *Hin*fI 1 μl, 10X *Hin*fI buffer 2 μl, PCR template 7-8 μl, add ultrapure water to 20 μl, kept in 37˚C water for 8 h. The fragments digested by *Hin*fI were electrophoresed in a 3% agarose gel. Genotypes were determined by the results of various bands though UV light (c150; Azure Biosystems, Inc.).

Western blot analysis and SDS-PAGE. Placental tissue total protein was extracted by RIPA with phenylmethylsulfonyl fluoride (PMSF) (Beyotime Institute of Biotechnology, Shanghai, China). Then put into homogenizer to grind into tissue homogenate and the supernatant was collected and centrifuged at 14,000 x g at 4˚C for 5 min. The protein concentration was quantified by BCA assay. Then, samples containing 50 μg were separated by polyacrylamide gel electrophoresis with a 10% separating gel (pH 8.8) and a 5% stacking gel (pH 6.8) (Beyotime Institute of Biotechnology) and the proteins were transferred onto PVDF membranes (Millipore, Billerica, MA, USA). Membranes were incubated with primary antibody: goat anti-human SHBG polyclonal antibody (INC sc-32468, 1:2,000 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and GAPDH monoclonal antibody (60004-1-lg, 1:10,000 dilution; Proteintech, Wuhan, China) overnight

at 4˚C after blocking in 5% skim milk for 2 h. Following three washes with 1X Tris-buffered saline containing 0.1% Tween-20 (TBST), the membranes were incubated in secondary antibody: HBP-conjugated rabbit anti-goat IgG (Ab-204-01, 1:5,000 dilution; Vazyme, Nanjing, China) and peroxidase-conjugated AffiniPure goat anti-mouse IgG (SA00001-1, 1:2,000 dilution; Proteintech) for 120 min then room temperature for 2 h. Immune complexes were detected with enhanced chemiluminescence (ECL) (Beyotime Institute of Biotechnology). A bioanalytical imaging system (c300; Azure Biosystems, Inc.) was used to catch the bands. All experiments were repeated at least three times. Data were expressed as a ratio of SHBG gray value to GAPDH (ImageJ; National Institutes of Health, Bethesda, MA, USA).

Cell culture. The placenta villus trophoblast cells HTR8-SVneo were obtained from Canada Queen's University and cultured conventionally with RPMI-1640 medium, supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, Carlsbad, CA, USA). Standard culture condition: 37˚C, 5% humidified CO_2 incubator. Cell culture supplies were purchased from the Greiner Bio-One GmbH (Frickenhausen, Germany), trypsin (Gibco).

Transfection. The recombinant lentivirus was designed and synthesized with all of the SBHG genetic sequence labeled by green fluorescence (GenePharma, Shanghai, China). For this part, HTR8-SVneo cells were divided into ten flasks, some of which had upregulated SHBG expression. A and B were the normal groups (without transfection), C and D were the blank control groups (transfected with empty virus LV-5). E-G were negative control groups (respectively transfected with SHBG rs6259 Asp-1, rs6259 Asp-2 and rs6259 Asp-3). H-J were the positive transfection group (respectively transfected with SHBG rs6259 Asn-1, rs6259 Asn-2 and rs6259 Asn-3).

One day before transfection, cells were cultured with 90% RPMI-1640+10% FBS and seeded in 25 cm^2 flasks (250-500x10^3 cells/flasks), cultivated at 37˚C, in 5% CO_2 incubator. Twenty-four hours later, the cell growth reached 30-40%, the medium was replaced and the cells were transfected with the lentivirus. Cells were incubated at 37˚C, morphological structure was observed at 12 h and the culture media was changed at 24 h. After 96 h, transfection efficiency was determined by using fluorescence imaging. Then, the cells were collected, SHBG mRNA and protein were assayed via western blot analysis and RT-qPCR.

Gene expression analysis by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total SHBG mRNA was extracted by TRIzol reagent (Invitrogen Life Technologies) from transfected cells according to the manufacturer's instructions. Amplification of SHGB cDNA fragment was divided into two parts: i) removal of extraneous DNA contamination: 1 μl RNA template, 2 μl 5X g DNA Erase buffer, 1 μl g DNA Erase buffer, 6 μl RNase Free dH_2O, at 42˚C for 2 min, then for 4˚C; ii) cDNA production: 4 μl 5X g Premix Script buffer 2, 1 μl Premix Script RT enzyme mix, 1 μl RT Primix Mix and 4 μl RNase Free dH_2O into reaction volume from step 1, at 37˚C for 15 min, at 85˚C for 5 sec, then for 4˚C (no. RR047A; Takara Bio, Inc.).

Table I. Clinical characteristics of normal and GDM pregnant women.

Characteristics	GDM group	Control group	P-value
Numbers	180	210	
Age (years)	31.59±3.89	31.73±4.46	0.735[b]
Fasting blood glucose	5.04±0.86	4.32±0.44	0.000[a]
Gestational weeks	38.69±1.13	38.83±0.79	0.141[b]
Childbearing history	0.10±0.31	0.16±0.37	0.056[b]
Birth weight (g)	3445.6±415.1	3394.5±469.1	0.308[b]

Independent samples t-test. [a]P<0.05, the difference of fasting blood glucose between control group and GDM group is statistically significant. [b]P>0.05, the differences are not statistically significant. GDM, gestational diabetes mellitus.

PCR primer sequences were as follows: SHBG mRNA forward primer, 5'-CCTCACCAAGATCACAAAAA-3' and reverse primer, 5'-TCTCGAAGTCCCAGCATAAACC-3', giving a fragment length of 120 bp; β-actin forward primer, 5'-AGCACAATGAAGATCAAGATCAT-3' and reverse primer, 5'-ACTCGTCATACTCCTGCTTGC-3', giving a fragment length of 127 bp (Invitrogen Life Technologis). Real-time PCR amplification was carried out in 7500 fast thermocycler (Life Technologies) as follows: 10 μl SYBR Premix Ex Taq, 6 μl RNase Free dH_2O, 1 μl each of SHBG and β-actin forward and reverse primers (10 μM), 2 μl cDNA template. Conditions were: 95˚C for 5 min (95˚C for 10 sec, 60˚C for 30 sec) 40 (no. RR820A; Takara Bio, Inc.). Gene expression was determined by $2^{-\Delta\Delta Ct}$, where $\Delta Ct = (Ct_{SHBG} - Ct_{GAPDH})$ and Ct is the threshold cycle.

Transfected HTR8-SVneo cell SHBG detection by western blot analysis. Expression of SHBG was tested and analyzed in different transfected groups with the same approach as placental tissue.

Statistical analysis. The direct counting method for % calculation of the gene frequency. The differences with alleles and genotypes distribution compared with Chi-square statistics, P<0.05 was considered statistically significant. Hardy-Weinberg balance check (HWE) the reliability of the survey data (gene frequency, estimation accuracy and reliability) and disease risk with rs6259 through the SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) (23). The correlation between SHBG levels and genotypes were evaluated by Pearson's correlation coefficient. Logistic regression models were used to analyse the correlation of genotype and disease with odds ratios (ORs) and 95% confidence intervals (95% CIs). T-test and LSD t-test were used with α=0.05 considered to be significant. All analyses used SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA).

Results

Clinical data analyses. The statistics and analysis of population characteristics are depicted in Table I, with the

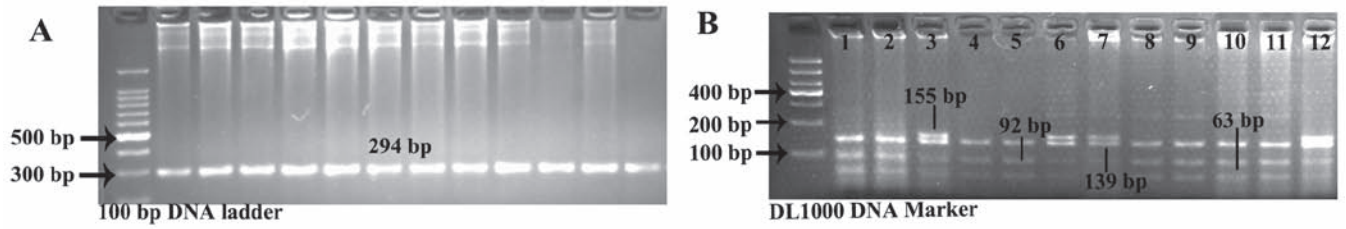

Figure 1. (A) Sex hormone-binding globulin (SHBG) rs6259 fragment amplification by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR fragments, 294 bp. (B) Results after restriction enzyme digestion. The GG genotype was constituted by segments of 139, 92 and 63 bp, while the AA genotype was characterized by segments of 155 and 139 bp and the GA genotype was characterized by segments of 155, 139, 92 and 63 bp. Lanes 1, 2, 4, 5, 8, 9, 10, 11: GG homozygote (Asp/Asp); lanes 3, 6, 12: GA heterozygote (Asn/Asp); lane 7, AA homozygote (Asn/Asn).

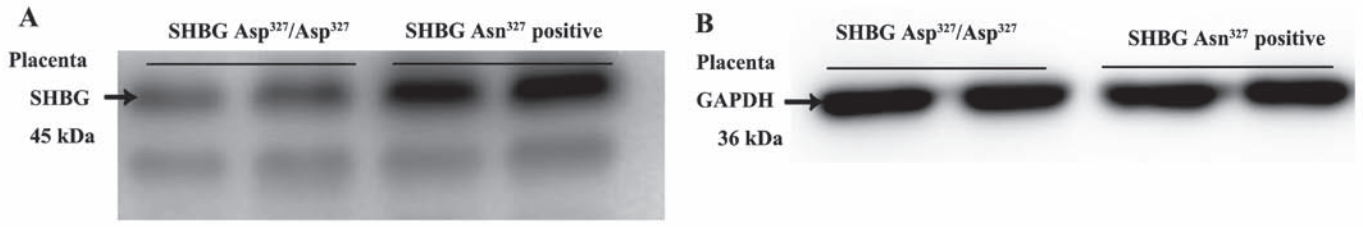

Figure 2. (A) Placenta tissue sex hormone-binding globulin (SHBG) protein expression in different genotype groups by western blot analysis. (B) Placenta tissue GAPDH protein expression in different genotype groups by western blot analysis.

mean ± standard deviation. The differences of age distribution, gestational weeks, childbearing history and birth weight had no significant difference, but statistically significant difference wasfound in fasting blood glucose between normal and GDM pregnant womean (P<0.05).

Placenta SHBG gene rs6259 allele frequencies and genotype distribution. PCR fragment amplification is shown in Fig. 1A. Three genotypes were detected in two groups: the GG genotype represented three segments, while the AA genotype was characterized by two parts and GA genotype was constituted by four segments (Fig. 1B). SHEsis online software detected the Hardy-Weinberg balance, control group: χ^2=0.469, P=0.493; GDM group: χ^2=0.141, P=0.707. The gene frequency conforms to the laws of genetic balance constantly (P>0.05). Allelic frequencies and genotype distributions are given in Table II. Because of the low proportion of AA homozygotes, they were considered together with the GA heterozygotes. In the GDM pregnancy group, 14.17% had the variant gene, higher than the control group. Compared with the control group, the decreased tendencies were observed in the GDM group with AA and GA group. The result [odd ratio <1, 95% CI=(0-1)] shows that rs6259 is a protective factor for decreased risk of GDM (P=0.043). Pearson's p=0.707 confirms that rs6259 is highly correlated with the morbidity of GDM.

SHBG expression of different genotypes in placental tissues. Following the results of PCR-RFLP and enzyme digestion, the placental tissues were divided into two groups. As shown in Fig. 2, the SHBG concentration of the GA heterozygote and AA homozygote (SHBG Asn^{327} positive group) are significantly higher than the GG homozygote (SHBG Asp^{327}/Asp^{327} group). The difference between the two groups was statistically significant (t=2.176, P=0.035 <0.001).

Table II. Allele and genotypic frequencies of SHBG $Asp^{327}Asn$ polymorphism in placenta.

	Control group (n=210)		GDM group (n=180)		
Frequencies	n	(%)	n	(%)	P-value
Allele					
A (Asn)	86	20.48	51	14.17	
G (Asp)	334	79.52	309	85.83	0.021[a]
Genotype					
GG (Asp/Asp)	131	62.38	132	73.33	0.021[b]
GA+AA	79	37.62	48	26.67	0.021[c]
GA (Asp/Asn)	72	34.29	45	25	0.046[d]
AA (Asn/Asn)	7	3.33	3	1.67	0.299[e]

Chi-square test, P<0.05, the differences is statistically significant. [a]The difference of allele frequency between control group and GDM group is statistically significant. [b]The difference of GG homozygote distribution between control group and GDM group is statistically significant. [c]The difference of GA+AA genotype distribution between control group and GDM group is statistically significant. SHBG, sex hormone-binding globulin; GDM, gestational diabetes mellitus.

HTR8-SVneo cell transfection. HTR8-SVneo cells were incubated in 5% CO_2, 37˚C incubator. After repeated exploration the optimal transfection conditions were: MOI=80, 5 μg/ml polybrene, which were successfully transfected with recombinant lentivirus and upregulation of SHBG expression (Fig. 3).

Recombinant intracellular SHBG protein assay. Western blot analysis and gel image software analysis demonstrated that the

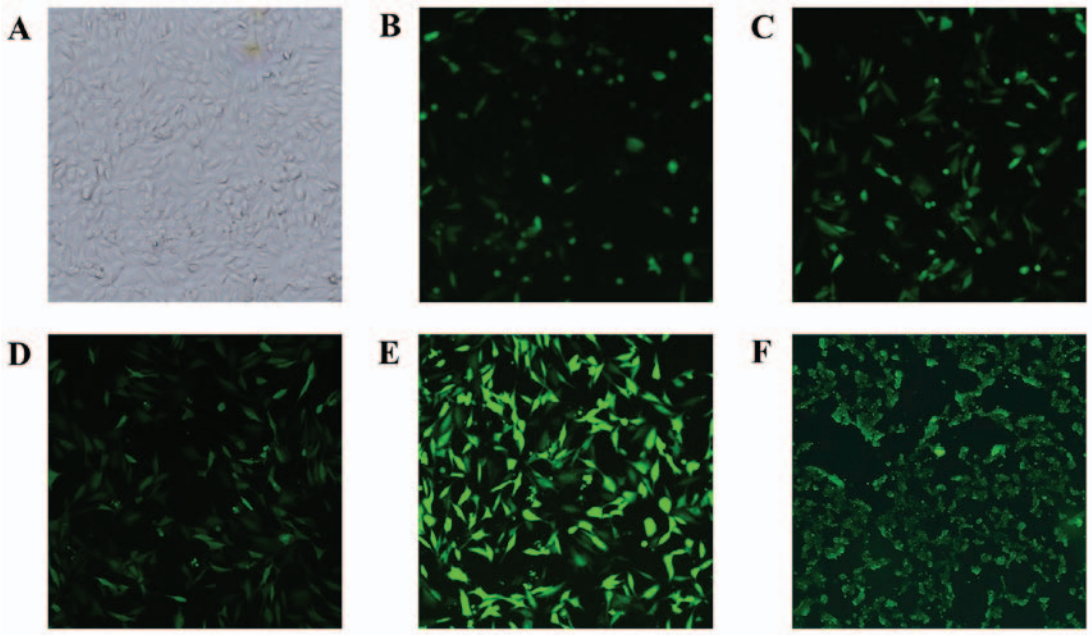

Figure 3. (A) Normal placenta villus trophoblast cells HTR8-SVneo. (B) HTR8-SVneo cells transfected with recombinant lentivirus 96 h (MOI=20, 5 μg/ml polybrene). (C) MOI=40, 5 μg/ml polybrene. (D) MOI=60, 5 μg/ml polybrene. (E) MOI=80, 5 μg/ml polybrene. (F) MOI=100.

Table III. The result of SHBG protein and mRNA in different groups of transfected cells.

A, Analysis of SHBG protein and GAPDH gray ratio in different transfected HTR8-SVneo cells

LSD t-test	Groups	P-value	95% CI
NG	LV-5	0.950[a]	-0.225-0.240
	rs6259 Asp	0.003[b]	0.116-0.560
	rs6259 Asn	0.000[c]	0.463-0.907
rs6259 Asn	rs6259 Asp	0.000[d]	0.148-0.545
LV-5 group	rs6259 Asp	0.002[e]	0.136-0.557
	rs6259 Asn	0.000[f]	0.482-0.902

B, Analysis of SHBG mRNA transcription in different transfected HTR8-SVneo cells

LSD t-test	Groups	P-value	95% CI
NG	LV-5	0.201[a]	-0.413-1.890
	rs6259 Asp	0.017[b]	0.272-2.575
	rs6259 Asn	0.000[c]	5.660-7.964
rs6259 Asn	rs6259 Asp	0.000[d]	4.237-6.541
LV-5 group	rs6259 Asp	0.001[e]	1.011-3.314
	rs6259 Asn	0.000[f]	6.399-8.703

LSD t-test, P<0.05, the differences have statistically significant. SHBG, sex hormone-binding globulin; CI, confidence interval.

positive transfection groups showed an increased SHBG level over negative control groups (t=2.675, P=0.011 <0.001), blank control and normal groups (P<0.001). There was no significant difference between the blank control group and the normal group (Table IIIA; Figs. 4 and 5).

Detection the SHBG mRNA in transfected HTR8-SVneo cells. According to the LDS-t analysis there were no significant differences between the blank control group and normal cell group (P>0.05), in the positive transfection groups a higher average $2^{-\Delta\Delta Ct}$ for SHBG mRNA were found than the negative control groups (P<0.001), blank control groups (P<0.001) and normal cell groups (P<0.001), the differences were statistically significant (Table IIIB; Fig. 5).

Discussion

The gene encoding human SHBG is located on chromosome 17p12-13 and is composed of 8 exons and 7 introns (Gene ID, 6462). The length of SHBG gene is 3.2 kb and it encodes a polypeptide of 402 amino acids. Human SHBG gene is prone to gene mutation, deletion and recombination. Because of its fragility and volatility, it is more likely to accept foreign interference (24,25). The polymorphic and the protein level changes may lead to special expression in certain diseases (26), such as IR damage (7,27), DM (7,23), polycystic ovary syndrome (21), osteoporosis (28), hirsutism (11), and even some hormone-dependent tumors, such as breast (16,20), ovarian (17), endometrial (18) and prostate cancers (29,30). rs6259 is one of the functional SHBG SNPs, which leads to an amino acid substitution of asparagine for aspartic acid at locus 327 ($Asp^{327}Asn$, D327N) in the SHBG polypeptide (11).

In our study, 180 pregnant women with GDM and 210 healthy pregnant women were chosen as participants in the program at the same period. The distributions of the genotypes and the allele frequencies had obvious differences in two groups. In GDM group, the frequency of the mutation gene and genotypes

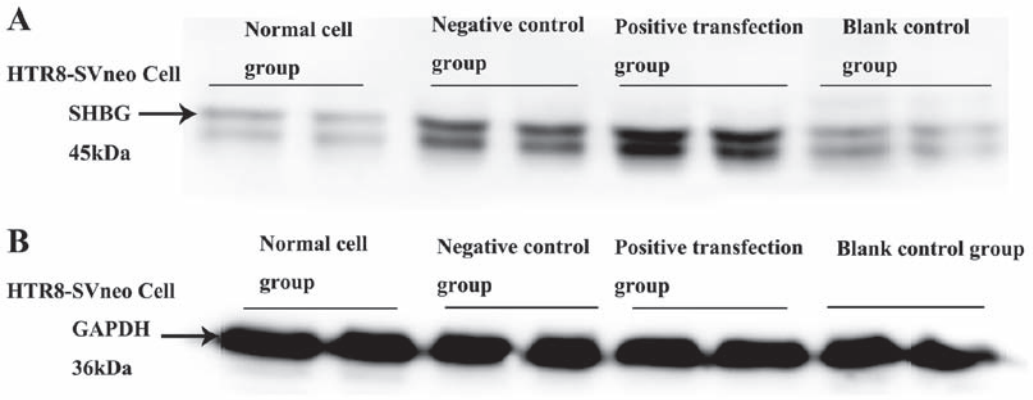

Figure 4. (A) Expression of sex hormone-binding globulin (SHBG) in each group of HTR8-SVneo cells. (B) Expression of GAPDH in each group of HTR8-SVneo cells.

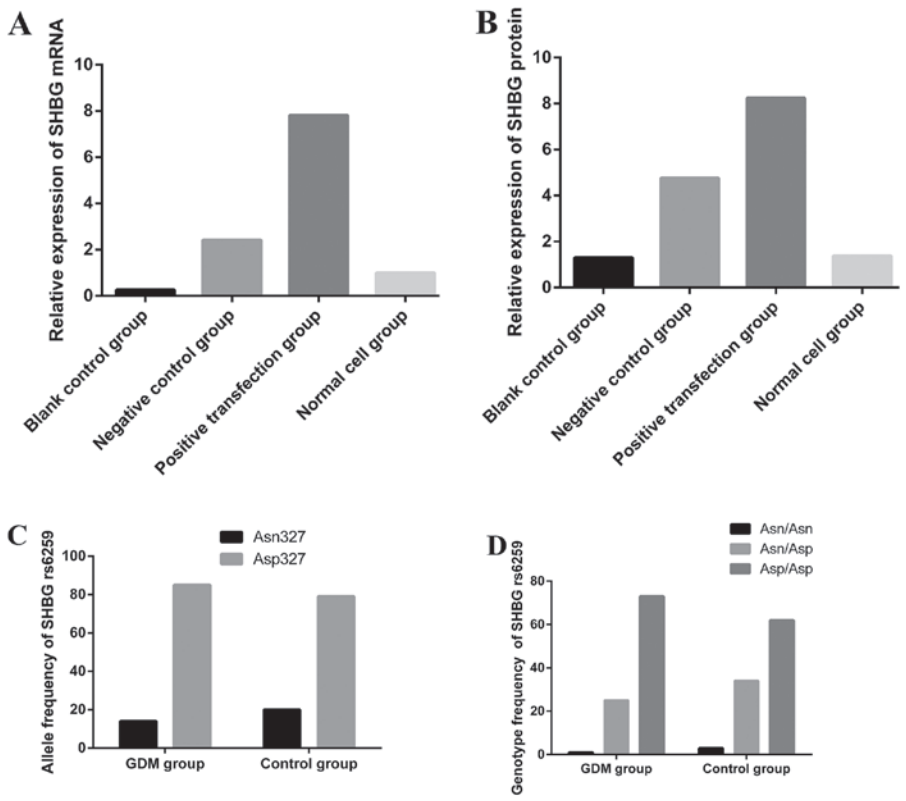

Figure 5. (A) The relative expression of sex hormone-binding globulin (SHBG) mRNA ($2^{-\Delta\Delta Ct}$), the positive transfection group led to an obviously higher tendency than the negative control group, the normal group and the blank control group. (B) The relative expression of SHBG (ratio of gray value of SHBG and GAPDH), the positive transfection group was obviously higher than negative control group, the normal group and the blank control group. (C) Distribution of SHBG rs6259 alleles (Asn and Asp) between gestational diabetes mellitus (GDM) and control group. (D) Frequency differences between GDM and control group.

showed decreasing trends. The frequency of the variant allele A is higher in healthy pregnant women of northeast China than in healthy Caucasian and African Americans, confirming the racial diversity in the distribution of this mutation.

Then, we measured SHBG level in each group. We discovered the differences between the two groups with disparate alleles (Asp^{327} or Asn^{327}). Through many repeats, we determined that the variant allele Asn^{327} have a strong connection with the added tendency of SHBG in the placenta organ. So, considering the result of genotypes screen, we speculated that Asn^{327} may lead to a reduced risk of GDM.

To clarify the mechanism involved, placental villi trophoblastic cells were cultivated continuously *in vitro*. With repeatedly recombinant lentivirus transfection, we found that both SHBG protein and SHBG mRNA in transfected HTR8-SVneo cells carried the mutant allele leading to higher level than others without mutation. Excluding other possible interference factors, the strong correlation between SHBG rs6259 variant allele A and SHBG transcription and translation was confirmed. It may be a genetic molecular basis of the pathogenesis of GDM.

It is well-known that type 2 diabetes is an endocrine disease with obvious genetic predisposition. If one of the parents has

type 2 diabetes, genetic odds of the offspring is between 1/7 and 1/13, but if both parents are affected, the genetic probability is increased to 1/2. Our preliminary experiment assessed the relevance between placental SHBG and GDM (8-10). The placenta has the same genetic material as the fetus, therefore, we anticipate to make a preliminary prediction for the possibility that the next generation achieve insulin resistance and type 2 diabetes, by testing the SHBG level and SHBG rs6259.

In conclusion, the findings of the present study support the result that SHBG SNP rs6259 is closely associated with placental SHBG levels. The mutant Asn^{327} allele may affect the transcription of SHBG mRNA and activity of the SHBG protein by some mechanisms. Associated with the SHBG rs6259 variant allele A, SHBG protein levels improved in placenta, the risk and progress of GDM may be delayed. SHBG rs6259 and SHBG are protective factors in pathogenesis of GDM, and may possiby be used to deduce the risk of offspring suffering from diabetes in the future.

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Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XZ contributed to manuscript writing, data collection and data analysis. LS contributed to data collection and financial support. ZJ contributed to project development.

Ethics approval and consent to participate

This study was in accordance with the ethical standards of the Shengjing Hospital of China Medical University and the ethical standards of the 1964 Helsinki declaration and its later amendments. All individual participants included in the study provided informed consents.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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