Polymorphisms of TGF-β1 and TGF-β3 in Chinese women with Gestational Diabetes Mellitus

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Abstract

Background: Gestational diabetes mellitus (GDM) is a pregnancy-specific carbohydrate intolerance Which can cause a large number of perinatal and postpartum complications. The members of Transforming growth factor-β (TGF-β) superfamily play key roles in the homeostasis of pancreatic β-cell and may involve in the development of GDM. This study aimed to explore the association between the polymorphisms of TGF-β1, TGF-β3 and the risk to GDM in Chinese women.

Methods: This study included 919 GDM patients (464 with preeclampsia and 455 without preeclampsia) and 1177 healthy pregnant women. TaqMan allelic discrimination real-Time PCR was used to genotype the TGF-β1 (rs4803455) and TGF-β3 (rs2284792 and rs3917201). The Hardy-Weinberg equilibrium (HWE) was evaluated by chi-square test.

Results: An increased frequency of TGF-β3 rs2284792 AA and AG
genotype carriers was founded in GDM patients (AA vs. AG+GG: $\chi^2=6.314$, $P=0.012$, OR=1.270, 95%CI 1.054-1.530; AG vs. GG+AA: $\chi^2=8.545$, $P=0.003$, OR=0.773, 95%CI 0.650-0.919). But there were no significant differences in the distribution of $TGF-\beta 1$ rs4803455 and $TGF-\beta 3$ rs3917201 between GDM and healthy women. And no significant differences were found in allele and genotype frequencies among GDM patients with preeclampsia (PE).

**Conclusions:** The AA and AG genotype of $TGF-\beta 3$ rs2284792 polymorphism may be significantly associated with increased risk of GDM in Chinese population.

**Keywords:** polymorphism; GDM; PE; TGF-\beta1; TGF-\beta3

**Introduction**

GDM is the most common maternal metabolic disturbance that is defined as glucose intolerance of variable severity with onset or first detection during pregnancy[1, 2]. The prevalence of GDM varies from 1%-22% of all pregnancies depending on different populations and diagnostic criteria[3-5]. GDM not only increases the risk of maternal and fetal perinatal complications, but also has long-term adverse consequences for offspring [6, 7]. The most familiar complication following GDM is preeclampsia (PE) which shares common clinical risk factors with GDM such as obesity, advanced maternal age and diabetes[8]. GDM is characterized by increased insulin resistance and defective insulin
secretion which is due to the inability of pancreatic β cells[9]. However, the etiology is complex due to disordered metabolism and intrauterine environment during pregnancy. Extensive efforts have been made to explore the pathogenesis and to find new targets for prediction of GDM[9-11].

In recent years, the role of genetic factors in the pathogenesis of GDM has been increasingly investigated. The major genetic studies of GDM are candidate gene studies, which have revealed that some single nucleotide polymorphisms (SNPs) in cytokine genes are associated with susceptibility to GDM [12, 13]. SNPs within the coding and signal sequences can affect gene transcriptional activity, and then change the production of proteins[14]. Several studies have reported that altered cytokines expression are related to the severity and progression of the GDM[15, 16]. Therefore, the cytokine gene with positive SNP loci may be a pregnancy biomarker for screening GDM.

TGF-β1 and TGF-β3 belong to TGF-β isoforms and have differential expression in the human endometrium and placenta[17]. Both of them contribute to normal homeostasis of pancreas and insulin action[18]. The enhanced expression of TGF-β1 induced by hyperglycemia was detected in individuals with GDM[19, 20]. Although there is no direct relation between TGF-β3 and GDM, TGF-β3 participates in many GDM complications such as preeclampsia and pregnancy-induced
hypertension[21]. Three tag SNPs (rs4803455, rs2284792, and rs3917201), located in introns of \( TGF-\beta 1 \) and \( TGF-\beta 3 \) locus respectively, can affect the transcriptional activity and change the expression of proteins[22-24]. Therefore, we supposed that these three SNPs might be target SNPs, and try to investigate the relationship between polymorphisms of \( TGF-\beta 1 \), \( TGF-\beta 3 \) and the risk of GDM.

**Subjects and methods**

**Subjects**

This study was conducted based on 919 pregnant women with GDM and 1177 healthy pregnant women with normal glucose tolerance, recruited from the clinical pregnancy registries at the Affiliated Hospital of Qingdao University, People’s Hospital of Liaocheng City and People's Hospital of Linyi City. Informed consent was issued and signed by all subjects and all investigations were approved by the ethics committee of the Affiliated Hospital of Qingdao University.

All the participants underwent a 75 g oral glucose tolerance test (OGTT) at 24–28 weeks' gestation. The diagnosis of GDM was based on the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria when one of the following plasma glucose values in the OGTT was met or exceeded, fasting plasma glucose 92 mg/dl (5.1 mmol/l), 1h plasma glucose 180 mg/dl (10.0 mmol/l) and 2h plasma glucose 153 mg/dl (8.5 mmol/l). Plasma glucose during OGTT of the follow-up study
was measured by enzymatic hexokinase photometric assay. Exclusion criteria included heart diseases, chronic hypertension, diabetes mellitus, thyroid diseases, kidney disorders, abnormal liver function, twin or multiple pregnancies, as well as artificial fecundation in the present gestation. Besides, 919 GDM patients were categorized into 455 without PE and 464 with PE which was determined on the base of the questionnaire, clinical features, and data. A newly onset of hypertension (≥140/90 mmHg) with proteinuria C of 300mg or higher in 24-hour after 20 weeks of gestation was diagnosed as PE.

Methods

Genomic DNA was extracted from peripheral venous blood using the Qiagen blood DNA extraction kit (Qiagen, Hilden, Germany). TaqMan allelic discrimination real-time PCR (Life Technologies, Grand Island, NY, USA) was used to genotype the polymorphisms of rs4803455 in TGF-β1, rs2284792 and rs3917201 in TGF-β3. The TaqMan probes and primers were designed by Applied Bio-systems or Life Technologies (New York, USA). TGF-β1 and TGF-β3 were amplified using the following primers:

5’-GCTGCAAAACATTCTGGGTTT-3’ for TGF-β1 rs4803455, 5’-GGGTGAGGACCAGGGGAATCT-3’ for TGF-β3 rs2284792 and 5’-CGCCTCAAGAAGCAGAAGGAT-3’ for TGF-β3 rs3917201. Reaction volume was 25μl: 1.25μL 20 × SNP Genotyping Assay, 12.5μL 2 × PCR Master Mix, and 11.25μL DNA and DNase-free water. 1000™ Thermal
cycler and CFX96™ Real-time system (Bio-Rad, California, USA) were carried out to amplifications as following conditions: 95°C for 3 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. The fluorescent signals from VIC/FAM-labeled probes were detected for each cycle. Discrimination of genotypes was conducted with BioRad CFX manager 3.0 software.

**Statistical analysis**

Statistical software package IBM SPSS 22.0 (SPSS Inc., Chicago, IL, USA) was used to manipulate all data. Student’s t-test was utilized to compare the demographic and clinical characteristics of cases and controls. An analysis of variance (ANOVA) was used to conduct the genotype-phenotype analysis. A chi-square test was performed to assess the HWE in the controls. Allelic and genotypic distributions were enrolled in the comparison by using Pearson’s $\chi^2$ test which was substituted with Fisher’s exact test when expected values were below 5. $P < 0.05$ (two-sided) was considered to represent statistically significance. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to reveal the relative risk degree. A $P$-value $< 0.05$ (two-sided) was taken as statistical significance for all statistical analyses.

**RESULTS**

**Demographic and clinical characteristics of GDM and controls**

Subjects were categorized into 919 GDM patients and 1177 controls.
Demographic and clinical data of different groups were summarized in the supplemental table.

Both groups had similar age distribution, times of gravidity, and number of abortions. The mean age of cases and controls was 30.71±4.18 and 30.75±4.21 years old. However, in GDM group, weeks of admission and delivery intended to be earlier (P<0.001) and the weight gain of newborns was heavier than in the control group as expected (P<0.001).

**TGF-β1 and TGF-β3 polymorphism analysis**

The subjects of the control group enrolled in this study were in accordance with HWE for these SNPs and displayed a group representative at the significance level of P > 0.05.

The distributions of the genotypes and alleles in GDM cases and controls were reported in Table 1. We observed a statistically significant difference between GDM and healthy women in the frequencies of TGF-β3 rs2284792 (χ² =9.064, P =0.011). However, no statistical differences were detected either in TGF-β1 rs4803455 or in TGF-β3 rs3917201 between two groups in terms of genotypic frequencies. As shown in Table 1, the allelic frequencies of rs2284792 between two groups were not obviously different (χ² =1.592, P =0.207, OR=1.082, 95%CI 0.957-1.224).

When categorized into three models (AA vs AG+GG, GG vs AG+AA and AG vs GG+AA), there was a significant difference between these two groups (For AA vs AG+GG model, χ²=6.314, P=0.012, OR 1.270, 95%CI
1.054-1.530; For AG vs GG+AA model, $\chi^2=8.545$, $P=0.003$, OR=0.773, 95% CI 0.650-0.919). Consistently, allelic frequencies of TGF-β1 rs4803455 or TGF-β3 rs3917201 were statistically insignificant.

**TGF-β1 and TGF-β3 polymorphism analysis between GDM patients with and without PE**

To further study the association between variants of the three SNPs and complications, samples were categorized into GDM cases with and without PE. The distributions of the genotypes and alleles in GDM patients with and without PE are shown in Table 2 and Table 3.

In GDM cases without PE group, the statistical difference between cases and controls in genotypic distributions of TGF-β3 rs2284792 was observed ($\chi^2=9.774$, $P =0.008$). Also, the same was found for allelic frequencies in AA vs AG+GG ($\chi^2=8.476$, $P =0.004$ OR=1.427, 95% CI 1.122-1.813) and AG vs GG+AA ($\chi^2=7.842$, $P =0.005$ OR=0.734, 95% CI 0.590-0.912). In contrast to TGF-β3 rs2284792, no obvious difference was found in either the genotypic distributions or allelic frequencies of TGF-β1 rs4803455 and TGF-β3 rs3917201 among GDM only cases.

In GDM cases with PE group, however, no obvious difference was found in either the genotypic distributions or allelic frequencies of three SNPs (for rs4803455, $\chi^2=1.266$, $P =0.531$ by genotype, $\chi^2 =0.069$, $P =0.793$, OR =1.021, 95% CI 0.873-1.194 by allele; when for rs2284792, $\chi^2=3.619$, $P =0.164$ by genotype, $\chi^2 =0.021$, $P =0.885$, OR =1.011, 95% CI
0.867-1.1791 by allele; and for rs3917201, $\chi^2 = 0.359$, $P = 0.836$ by genotype, $\chi^2 = 0.015$, $P = 0.903$, $OR = 1.009$, 95% CI 0.867-1.175 by allele).

Analysis of Genotype-Phenotype Relationship

Analysis of the relationship between the genotypes of TGF-β3 rs2284792 and demographic characteristics among total GDM patients was shown in Table 4. However, no statistical differences were found for the genotype-phenotype relationship of rs2284792.

DISCUSSION

GDM is characterized by varying degrees of hyperglycemia due to the inability of pancreatic β-cells to adequately respond to the increased insulin requirements during the second and third trimester[25, 26]. The etiology of GDM may be explained by many factors including cytokines, hormones, lifestyle as well as genetic disposition[27]. Nowadays, accumulating data suggest that genetic components play a key role in the development of GDM[28]. A Danish twin study indicated that the variation of the insulin secretion and action traits can be partially explained by genetic components[29]. In this study, we investigated the association of relatively frequently studied genetic variants for TGF-β isoforms with GDM in a Chinese population.

The most common genetic study of GDM is to find candidate genes that was previously based on biological plausibility[30]. Recently, genome-wide association analysis studies have been used to identify some
susceptibility genes associated with GDM[4]. The genetic variants of
candidate genes have been revealed to contribute to the risk of GDM. For
example, *Transcription factor 7-like 2* rs12255372 variant is suggested to
interact with adiposity to alter β-cell function in 132 Mexican-American
families with GDM[31]. Homozygosity for *Insulin receptor substrate-1*
G972R polymorphism might indicate an increased risk for GDM in Saudi
women[32]. Interestingly, many GDM associated candidate genes can
express cytokines that have been implicated in the inflammatory conditions
during pregnancy[33]. TGF-β isoforms are multifunctional factors that
regulate embryonic development, immunity, and epithelial homeostasis.
TGF-β1 and TGF-β3 have been used more extensively than TGF-β2[34].
With such attributes, we chose *TGF-β1* and *TGF-β3* as target genes and
tried to uncover the genetic disposition of GDM.

*TGF-β1* is reported to be a key cytokine in insulin resistance and
obesity. Over-expression of TGF-β1 can lead to decreased β-cell mass and
insulin secretion[35]. *TGF-β1* rs4803455 polymorphism is an A/C single-
nucleotide variation on chromosome 19q13.2 and can alter the expression
of insulin receptor substrate 2 associated with insulin resistant in GDM,
but not depending on its expression in the pathway[36]. Moreover, a
previous study suggested that *TGF-β1* rs4803455 showed the effectiveness
to capture the associations with cancer risk[37]. However, our data
revealed that *TGF-β1* rs4803455 was not a significant risk factor of GDM
in the Chinese Population. The difference between these studies could be attributed to the discordance of population genetic background. However, the finite sample size in these studies is another limiting factor to have a coincident conclusion.

This is the first study to show the relationship between the genetic polymorphism of TGF-β3 gene and GDM. Candidate SNPs previously described were chosen based on their location within the gene, and a tag SNP (rs2284792: A>G) selected with SNP picker using data from the Caucasian population was located within the introns of TGF-β3 [38]. Our studies revealed an effective association between the tag SNP rs2284792 and GDM risk. Besides, we confirmed that the A allele and the A allele-containing genotypes (AA and AG) were susceptible, while the G allele/GG genotype may be protective factors. TGF-βs in mammals exhibit many overlapping biological activities and appear interchangeable. TGF-β3 knock-in ameliorate inflammation due to TGF-β1 deficiency while promoting glucose tolerance[39]. Reduced TGF-β3 expression can cause hypertrophy and induce glucose intolerance[40]. Therefore, altered generation made by polymorphic variants in TGF-β3 may affect glucose homeostasis, thus leading to GDM.

GDM is a transient presentation of long-standing metabolic malfunction and may be expected to have an association with PE[41]. The pathophysiology of PE is characterized by endothelial dysfunction which
may be induced by down-regulation of TGF-β signaling. TGF-β isoforms were predisposed to have obvious susceptible associations with PE and were supposed as a biomarker for assessment of PE severity[42, 43]. TGF-β1 codon 10T/C was observed to have a higher frequency of T > C allele in Type 2 Diabetes Mellitus patients with hypertension[44]. A fetal TGF-β3 variant (rs11466414) is associated with preeclampsia in a predominantly Hispanic population[43]. In consideration of comparable clinical characteristics, we hypothesized that the variants of TGF-β isoforms may relate to the development of both disease conditions. Then, we analyzed TGF-β1 (rs4803455) and TGF-β3 (rs2284792 and rs3917201) polymorphisms among GDM cases with PE. However, no obvious difference was found in either the genotypic distributions or allelic frequencies among above three SNPs. The complexity of several pathogenic pathways including metabolic, immune, and endothelial dysfunction can account for the invalid assumption. Insulin resistance which is highly prevalent in patients with GDM can only partially explain the development of PE[45]. To sum up, TGF-β3 rs2284792 may be the independent effective genetic locus for GDM alone.

**CONCLUSIONS**

This study indicated that the AA and AG genotype rs2284792 polymorphism of TGF-β3 was associated with the increased risk of GDM. However, some evident shortcomings are the limited sample size and the
different ethnic origins. Furthermore, some environmental factors, such as
behavioral and pharmacological interventions, will be considered in our
future studies. All these studies highlight the need of long-term cohort
studies of women with GDM for ultimately improving pregnancy
outcomes.

**Abbreviations**

TGF-β: Transforming growth factor-β; GDM: Gestational diabetes
mellitus; HWE: Hardy-Weinberg equilibrium; PE: preeclampsia; SNPs:
Signal Nucleotide Polymorphisms; OGTT: oral glucose tolerance test; ORs:
Odds ratios; Cis: confidence intervals

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**Authors’ contributions**

Yinglei Xu: study design, protocol development; Cuijiao Wu, Mengmeng
Han, Jingli Wang, Rui Zhang: collecting clinical samples; Yinglei Xu
Chunlian Wei: data analysis; Yinglei Xu: writing the manuscript; Ying
Chen, Shiguo Liu: critical review of the manuscript; Shiguo Liu is
responsible for the integrity and the accuracy of the data analysis.

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**Availability of data and materials**

The original data used to support the findings of this study are available from the corresponding author upon request.

**Ethics approval and consent to participate**

Informed consent was issued and signed by all subjects and all investigations were approved by the ethics committee of the Affiliated Hospital of Qingdao University.

**Consent for publication**

Written informed consent for publication was obtained from all subjects.

**Conflict of interests**

The authors declare that they have no competing interests.

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