Association of Single Nucleotide Polymorphisms of the *IL-6*, *IL-10*, and *TNF- α* Genes with Susceptibility to Gestational Diabetes Mellitus

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Objective: To investigate any associations between the single nucleotide polymorphisms (SNPs) at the interleukin-6 (*IL-6*) rs1800796, interleukin-10 (*IL-10*) rs1800896, and the tumor necrosis factor-alpha (*TNF- α*) rs1800629 loci with gestational diabetes mellitus (GDM) susceptibility.

Materials and Methods: A total of 242 GDM patients and 242 healthy controls were enrolled in this study. The genotypes of the *IL-6* rs1800796, *IL-10* rs1800896, and *TNF- α* rs1800629 loci were analyzed by Sanger sequencing. Interactions among these SNPs were analyzed through multifactor dimensionality reduction.

Results: Women with the *IL-6* rs1800796 G allele had a higher GDM susceptibility risk than those with the C allele (odds ratio [OR]=1.44, 95% confidence interval [CI]: 1.10–1.90, $p=0.010$). Women with the *IL-10* rs1800896 C allele had a higher risk of GDM susceptibility than those with the T allele (OR=2.94, 95% CI: 1.87–4.63, $p<0.001$). Women with the *TNF- α* rs1800629 A allele had a higher risk of susceptibility to GDM than those with the G allele (OR=3.73, 95% CI: 2.25–6.18, $p<0.001$). The plasma levels of *IL-6* and *TNF- α* in GDM patients were significantly higher than those in the control group, and the levels of *IL-10* were significantly lower than those in the control group ($p<0.001$). Women with the *IL-6* rs1800796 CG/GG genotypes had higher plasma *IL-6* levels than those with the CC genotype ($p<0.05$). Women with the *IL-10* rs1800896 TT genotype had higher *IL-10* levels than those with the TC/CC genotypes ($p<0.05$), and those with the *TNF- α* rs1800629 GA/AA genotypes had higher *TNF- α* levels than those with the GG genotype ($p<0.05$).

Conclusion: The results of this study show that the *IL-6* rs1800796 G allele, the *IL-10* rs1800896 C allele, and the *TNF- α* rs1800629 A allele are significantly associated with an increased risk of susceptibility to GDM.

Keywords: gestational diabetes mellitus, *IL-6*, *IL-10*, *TNF- α* , single nucleotide polymorphism

Introduction

GESTATIONAL DIABETES MELLITUS (GDM) refers to a range of glucose metabolism abnormalities that occur or are first discovered during pregnancy. GDM is a special type of diabetes that can significantly increase the morbidity and mortality rates of pregnant women and their fetuses (Sacks, 2014; McIntyre *et al.*, 2019; Zhang *et al.*, 2019). The pathogenesis of GDM is not yet fully understood. The immunologic/inflammatory pathogenesis model is a prevalent topic. It is generally believed that insulin resistance and decreased insulin sensitivity, which can be caused by a myriad of different reasons, are the main causes of GDM (Yang *et al.*, 2013; Tang *et al.*, 2019; Feng *et al.*, 2020).

Recent studies have shown that interleukin-6 (*IL-6*) and tumor necrosis factor-alpha (*TNF- α*), as proinflammatory

factors, play important roles in development of insulin resistance and GDM (Nergiz *et al.*, 2014; Lacroix *et al.*, 2019; Rancourt *et al.*, 2020). Interleukin 10 (*IL-10*) is an important anti-inflammatory cytokine that can be synthesized and secreted by a variety of cells; it can inhibit production of inflammatory cytokines by a variety of inflammatory cells. *IL-10* not only plays an important regulatory role in the body's immune response but it also plays a wider role in many tissues and organs, including its participation in cell activation, proliferation, and differentiation (Laffer *et al.*, 2019; Zhang and Kuchroo, 2019). Studies have shown that plasma *IL-10* levels in GDM patients are significantly lower than in healthy controls and that low levels of *IL-10* and excess insulin resistance in GDM patients are negatively correlated, suggesting that low levels of *IL-10* may increase insulin resistance in GDM patients (Charles *et al.*, 2011; Kowalski *et al.*, 2011; Yang *et al.*, 2018).

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The *IL-6* gene is located on the human chromosome 7p15-21; it has a total length of 5 kb and consists of five exons and four introns. The gene has three transcription initiation sites (ATG) and three TATA sequences (TATA box). The non-coding region at the 5' end is ~300 bp upstream of the start site and contains most of the regulatory sequences for the *IL-6* gene expression, with its expression being mainly regulated at the transcriptional level. The human *IL-6* gene promoter region contains several polymorphisms that can affect its transcription (-597G/A, -572G/C, and -174G/C, etc.). The 180 to 123-bp region upstream of the *IL-6* gene promoter is a key region for *IL-6* gene transcription regulation (Ma *et al.*, 2011; Chang *et al.*, 2015). Revilla *et al.* (2002) found that the existence of *IL-6* gene polymorphisms causes differing susceptibilities to multiple diseases among individuals. The presence of a G/C single nucleotide polymorphism (SNP) at the 174th base upstream of the *IL-6* gene promoter can affect its transcription, and since the concentration of *IL-6* varies with the efficiency of its gene transcription, this allele affects the occurrence and development of numerous diseases.

The *IL-10* gene is located at q32 of human chromosome 1 and its expressed *IL-10* protein is a multifunctional, cellular immunosuppressive factor produced by Th2 cells; it is involved in inflammatory and immune responses and is currently recognized as an inflammatory and immune suppressive factor (Jin *et al.*, 2019; Wang *et al.*, 2019). There are multiple SNP sites in the *IL-10* gene, among which are 1082G/A, 819 C/T, and 592 C/A that are located within the promoter region and potentially could affect gene transcription and expression levels (Jin *et al.*, 2019; Kalani *et al.*, 2019; Shahriyari *et al.*, 2019).

The *TNF* genes are located in a 7-kb DNA sequence within the short arm of chromosome 6 inside the MHC class III gene region. They consist of *TNFA* and *TNFB*, encoding TNF- α and TNF- β , respectively, each containing four exons and three introns. There are many SNPs in the *TNF- α* promoter, including -1031 T/G, -863 C/A, -851 C/T, -419 G/C, -376 G/A, -308 G/A, and -238 G/A. Among them, rs1800629 (-308 G/A) is known to be related to the TNF- α transcriptional level as transcriptional factors bind more strongly to the A allele, resulting in higher plasma TNF- α levels—twofold higher than that of subjects with the G allele (Warzocha *et al.*, 1998).

In this study, we investigated the correlation between *IL-6* rs1800796, *IL-10* rs1800896, and *TNF- α* rs1800629 SNPs and their roles in regard to susceptibility to GDM.

Materials and Methods

Subjects

A total of 242 patients who were diagnosed with GDM after routine prenatal examination in our obstetrics and gynecology clinic from May 2017 to October 2019 were enrolled. The reference standard for a diagnosis of GDM is that of the International Association of the Diabetes and Pregnancy Study Groups criteria (International Association of Diabetes and Pregnancy Study Groups Consensus Panel 2010). According to the age and body mass index (BMI) matching of GDM patients, 242 healthy controls were selected. Those with chronic hypertension, diabetes, rheumatoid arthritis and other inflammatory diseases, cancer, and chronic renal insufficiency were excluded. Our research protocol was approved by the Medical Ethics Committee of Hangzhou Fufang Women's and Children's Hospital, and informed consent was obtained from all subjects.

Obtaining clinical data and blood biochemical data from subjects

The age, gestational age, BMI before pregnancy, BMI at birth, newborn body mass, and APGAR data were collected from patients' medical records, and the plasma glycosylated hemoglobin A1c (HbA1c) and glucose levels were measured at 0, 1, and 2 h during glucose stimulation.

Genotypic analyses

We used the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) to extract genomic DNA from the peripheral blood of the subjects. Sanger sequencing was used to analyze the genotypes of the *IL-6* rs1800796, *IL-10* rs1800896, and *TNF- α* rs1800629 loci. Genomic DNA samples from 20% of the subjects were randomly selected for repeated sequencing. The results of the sequencing runs were entirely consistent.

Enzyme-linked immunosorbent assay

We collected 3 mL of peripheral blood from the subjects, let it stand at room temperature for 1–2 h, centrifuged it at 3000 r/min for 5 min, removed the plasma, and stored it at -80°C. In accordance with the manufacturer's instructions, the enzyme-linked immunosorbent assay (ELISA) was used

TABLE 1. COMPARISON OF CLINICAL FEATURES AND BIOCHEMICAL INDICATORS OF GDM PATIENTS AND CONTROL GROUPS

Clinical features	GDM (n=242)	Control (n=242)	p
Age (years, mean $\pm$ SD)	29.51 $\pm$ 5.07	29.74 $\pm$ 4.90	0.61
Gestational weeks (mean $\pm$ SD)	38.26 $\pm$ 1.48	38.16 $\pm$ 0.82	0.36
BMI before pregnancy (kg/m ² , mean $\pm$ SD)	23.86 $\pm$ 2.06	23.80 $\pm$ 2.07	0.75
BMI at birth (kg/m ² , mean $\pm$ SD)	29.94 $\pm$ 2.23	29.67 $\pm$ 3.08	0.27
Newborn body mass (g, mean $\pm$ SD)	3321.11 $\pm$ 550.74	3292.95 $\pm$ 432.67	0.53
APGAR (mean $\pm$ SD)	9.81 $\pm$ 0.51	9.78 $\pm$ 0.43	0.49
HbA1c (% , mean $\pm$ SD)	5.54 $\pm$ 1.13	4.29 $\pm$ 0.96	<0.001
Zero-h plasma glucose (mM)	4.86 $\pm$ 0.69	4.32 $\pm$ 1.19	<0.001
One-h plasma glucose (mM)	10.16 $\pm$ 1.82	7.31 $\pm$ 1.55	<0.001
Two-h plasma glucose (mM)	8.88 $\pm$ 1.33	6.55 $\pm$ 1.28	<0.001

APGAR, activity, pulse, grimace, appearance, and respiration; BMI, body mass index; GDM, gestational diabetes mellitus; HbA1c, hemoglobin A1c; SD, standard deviation.

TABLE 2. IL-6 rs1800796, IL-10 rs1800896, AND TNF- α rs1800629 LOCUS GENOTYPES AND ALLELE FREQUENCIES

	GDM (n = 242)	Control (n = 242)	HWE p	OR (95% CI) ^a	p
IL-6 rs1800796					
CC	107 (44.21%)	133 (54.96%)	0.06	1.00	
CG	99 (40.91%)	85 (35.12%)		1.45 (0.98–2.13)	0.074
GG	36 (14.88%)	24 (9.92%)		1.86 (1.05–3.32)	0.046
CG+GG vs. CC				1.54 (1.08–2.20)	0.023
GG vs. CG+CC				1.59 (0.91–2.75)	0.129
C	313 (64.67%)	351 (72.52%)		1.00	
G	171 (35.33%)	133 (27.48%)		1.44 (1.10–1.90)	0.010
IL-10 rs1800896					
TT	184 (76.03%)	216 (89.26%)	0.16	1.00	
TC	42 (17.36%)	24 (9.92%)		2.05 (1.20–3.52)	0.012
CC	16 (6.61%)	2 (0.83%)		1.93 (1.37–2.15)	0.001
TC+CC vs. TT				2.62 (1.58–4.33)	<0.001
CC vs. TC+TT				1.83 (1.30–2.04)	0.002
T	410 (84.71%)	456 (94.21%)		1.00	
C	74 (15.29%)	28 (5.79%)		2.94 (1.87–4.63)	<0.001
TNF- α rs1800629					
GG	191 (78.93%)	222 (91.74%)	0.40	1.00	
GA	32 (13.22%)	19 (7.85%)		1.96 (1.08–3.57)	0.038
AA	19 (7.85%)	1 (0.41%)		2.05 (1.56–2.17)	<0.001
GA+AA vs. GG				1.55 (1.26–1.82)	<0.001
AA vs. GA+GG				1.98 (1.50–2.08)	<0.001
G	414 (85.54%)	463 (95.66%)		1.00	
A	70 (14.46%)	21 (4.34%)		3.73 (2.25–6.18)	<0.001

^aAdjusted for age, gestational age, BMI before pregnancy, and BMI at birth.

CI, confidence interval; HWE, Hardy–Weinberg equilibrium; IL, interleukin; OR, odds ratio; TNF- α , tumor necrosis factor- α .

to detect levels of IL-6 (ab178013; Abcam, UK), IL-10 (ab133575; Abcam), and TNF- α (ab1793; Abcam) in plasma.

Statistical analyses

In this study, SPSS 20.0 (SPSS, Chicago, IL) was used for statistical analyses. Continuous variables were evaluated by the t-test and expressed as the mean $\pm$ standard deviation. n (%) represents a categorical variable. Hardy–Weinberg equilibrium (HWE) was evaluated using the χ^2 test for the statistical analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to analyze the correlation between the genotypic and allelic frequencies of IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 and GDM susceptibility and adjusted for the age, gestational week, BMI before pregnancy, and BMI at birth. In a multi-comparison analysis, the Bonferroni correction was used to correct the p -value, and $p < 0.0125$ ($0.05/4$) was considered to be significantly different. Multifactor dimensionality reduction (MDR) was used to analyze the IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 site interactions. The receiver operating curve (ROC) analysis was used to evaluate

the diagnostic value of plasma for IL-6, IL-10, and TNF- α in GDM. All tests were two-tailed, and $p < 0.05$ indicated a statistically significant difference.

Results

Subject clinical characteristics

The clinical signs and symptoms as well as biochemical indicators of the GDM patients and control group are shown in Table 1. There were no significant differences between the GDM patients and controls for age of patients, gestational weeks, BMI before pregnancy, BMI at birth, newborn body mass, and the activity, pulse, grimace, appearance, and respiration (APGAR) score (all p -values < 0.05). The levels of plasma HbA1c and glucose were significantly lower among the control group compared with the GDM patients ($p > 0.05$).

IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 and GDM susceptibility

The genotypic frequencies of the IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 loci in the control group of

TABLE 3. GMDR ANALYSIS OF IL-6 rs1800796, IL-10 rs1800896, AND TNF- α rs1800629 INTERACTIONS

	Accuracy	Sensitivity	Specificity	Precision	OR (95% CI)	p
rs1800896	56.61%	23.97%	89.26%	69.05%	2.62 (1.58–4.33)	<0.001
rs1800896, rs1800629	61.16%	40.08%	82.23%	69.29%	3.10 (2.04–4.70)	<0.001
rs1800796, rs1800896, rs1800629	61.36%	44.21%	78.51%	67.30%	2.90 (1.95–4.31)	<0.001

GMDR, generalized multifactor dimensionality reduction.

FIG. 1. MDR analysis of IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 interactions. **(A)** Graphical model. In each box, the *left bar* represents the number of GDM patients and the *right bar* represents the number of subjects in the control group. **(B)** Circle graph. The *bar boxes* represent the SNP loci and the percentage represents the effect of the SNP loci on GDM susceptibility. The larger the data, the greater the impact of the SNP loci on GDM susceptibility. The data on the *line* represent the strength of the interactions between SNPs. **(C)** Dendrogram. The closer the SNP site, the stronger the interaction. GDM, gestational diabetes mellitus; HbA1c, hemoglobin A1c; IL, interleukin; MDR, multifactor dimensionality reduction; SNP, single nucleotide polymorphism; TNF- α , tumor necrosis factor-alpha.

the study were in accordance with HWE ($p > 0.05$). The genotypic and allelic frequency distributions of IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 are shown in Table 2. The IL-6 rs1800796 GG genotype under the dominant model was associated with an increased sus-

ceptibility to GDM (OR = 1.86, 95% CI: 1.05–3.32, $p = 0.046$; OR = 1.54, 95% CI: 1.08–2.20, $p = 0.023$). Persons with the G allele have a higher risk of susceptibility to GDM than those with the C allele (OR = 1.44, 95% CI: 1.10–1.90, $p = 0.010$). At the IL-10 rs1800896 loci, the TC and CC genotypes under

TABLE 4. COMPARISON OF CLINICAL CHARACTERISTICS OF IL-6 rs1800796 LOCI WITH DIFFERENT GENOTYPES

	GDM		p	Control		p
Age (years)	29.89 $\pm$ 5.12	29.21 $\pm$ 5.03	0.30	29.73 $\pm$ 5.42	29.74 $\pm$ 4.20	0.99
Gestational weeks	38.26 $\pm$ 1.30	38.32 $\pm$ 1.57	0.75	38.21 $\pm$ 0.76	38.10 $\pm$ 0.88	0.30
BMI before pregnancy (kg/m ²)	23.95 $\pm$ 2.35	23.79 $\pm$ 1.79	0.56	23.79 $\pm$ 2.12	23.81 $\pm$ 2.01	0.94
BMI at birth (kg/m ²)	30.15 $\pm$ 2.21	29.76 $\pm$ 2.23	0.18	29.88 $\pm$ 3.12	29.41 $\pm$ 3.02	0.24
Newborn body mass (g)	3269.30 $\pm$ 518.09	3362.17 $\pm$ 573.89	0.19	3295.50 $\pm$ 429.05	3289.83 $\pm$ 439.01	0.92
APGAR	9.77 $\pm$ 0.56	9.82 $\pm$ 0.50	0.47	9.75 $\pm$ 0.47	9.82 $\pm$ 0.39	0.21
HbA1c (%)	5.63 $\pm$ 1.15	5.47 $\pm$ 1.12	0.28	4.20 $\pm$ 1.04	4.39 $\pm$ 0.86	0.12
Zero-h plasma glucose (mM)	4.81 $\pm$ 0.66	4.89 $\pm$ 0.72	0.37	4.25 $\pm$ 1.25	4.40 $\pm$ 1.12	0.33
One-h plasma glucose (mM)	10.09 $\pm$ 1.89	10.21 $\pm$ 1.77	0.61	7.37 $\pm$ 1.57	7.23 $\pm$ 1.53	0.49
Two-h plasma glucose (mM)	8.92 $\pm$ 1.39	8.84 $\pm$ 1.28	0.64	6.58 $\pm$ 1.26	6.51 $\pm$ 1.31	0.67

TABLE 5. COMPARISON OF CLINICAL CHARACTERISTICS OF IL-10 rs1800896 LOCI WITH DIFFERENT GENOTYPES

	GDM			Control		
	TT (n=184)	TC/CC (n=58)	p	TT (n=216)	TC/CC (n=26)	p
Age (years)	29.44±5.07	29.72±5.14	0.72	29.79±4.92	29.31±4.79	0.64
Gestational weeks	38.26±1.47	38.28±1.54	0.93	38.16±0.82	38.15±0.78	0.95
BMI before pregnancy (kg/m ²)	23.77±2.21	24.14±2.36	0.28	23.86±2.07	23.34±1.99	0.23
BMI at birth (kg/m ²)	29.91±2.21	30.02±2.31	0.74	29.72±3.11	29.24±2.75	0.45
Newborn body mass (g)	3324.99±552.10	3308.78±551.03	0.85	3269.41±423.17	3388.50±469.11	0.21
APGAR	9.80±0.52	9.78±0.56	0.80	9.78±0.44	9.81±0.40	0.74
HbA1c (%)	5.58±1.12	5.41±1.17	0.32	4.28±0.99	4.35±0.75	0.73
Zero-h plasma glucose (mM)	4.89±0.71	4.76±0.63	0.21	4.39±1.14	3.93±1.43	0.06
One-h plasma glucose (mM)	10.15±1.81	10.21±1.86	0.83	7.29±1.55	7.42±1.58	0.69
Two-h plasma glucose (mM)	8.83±1.32	9.02±1.36	0.34	6.57±1.28	6.35±1.26	0.41

the dominant and recessive models were significantly associated with an increased risk of susceptibility to GDM (OR=2.05, 95% CI: 1.20–3.52, $p=0.012$; OR=1.93, 95% CI: 1.37–2.15, $p=0.001$; OR=2.62, 95% CI: 1.58–4.33, $p<0.001$; OR=1.83, 95% CI: 1.30–2.04, $p=0.002$). Persons with the C allele have a higher risk of susceptibility to GDM than those with the T allele (OR=2.94, 95% CI: 1.87–4.63, $p<0.001$). Persons with TNF- α rs1800629 GA and AA genotypes have a higher risk of susceptibility to GDM than those with GG genotypes (OR=1.96, 95% CI: 1.08–3.57, $p=0.038$; OR=2.05, 95% CI: 1.56–2.17, $p<0.001$; OR=1.55, 95% CI: 1.26–1.82, $p<0.001$; OR=1.98, 95% CI: 1.50–2.08, $p<0.001$), and persons with the A allele have a higher risk of susceptibility to GDM than those with the G allele (OR=3.73, 95% CI: 2.25–6.18, $p<0.001$).

IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 interactions

The MDR analysis of IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 was designed to measure their interactions with respect to GDM susceptibility. The results of the accuracy (61.36%) and sensitivity (44.21%) of GDM predicted by the interactions among IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 are the highest and the specificity (78.51%) and precision (67.30%) are the lowest. (OR=2.91, 95% CI: 1.95–4.31, $p<0.001$) (Table 3). In this study, subjects with IL-6 rs1800796 CC, IL-10 rs1800896 TC, and TNF- α rs1800629 GG had the highest risk of GDM

susceptibility (OR=2.14, 95% CI: 1.85–2.65, $p=0.005$). The subjects with IL-6 rs1800796 CC, IL-10 rs1800896 TT, and TNF- α rs1800629 GG had the lowest risk of GDM susceptibility (OR=0.43, 95% CI: 0.35–0.67, $p=0.012$) (Fig. 1A). The cyclic chart analysis results showed that the effects of TNF- α rs1800629, IL-10 rs1800896, and IL-6 rs1800796 on susceptibility to GDM were sequentially reduced (Fig. 1B) and that there is a strong interaction between IL-6 rs1800796 and TNF- α rs1800629, followed by IL-10 rs1800896 (Fig. 1C).

Correlations between IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 alleles/genotypes and the clinical characteristics of subjects

We found that there were no differences in age, gestational weeks, BMI before pregnancy, BMI at birth, newborn body mass, plasma APGAR score, and glucose between GDM patients and the control group with different genotypes at IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 loci ($p>0.05$, Tables 4–6).

Plasma inflammatory factor levels

We used ELISAs to detect the plasma levels of IL-6, IL-10, and TNF- α in all subjects. The results showed that IL-6 plasma levels in GDM patients were significantly higher than those in the control group ($p<0.001$, Fig. 1A) and the area under the curve (AUC) of the working curve

TABLE 6. COMPARISON OF CLINICAL CHARACTERISTICS OF THE TNF- α rs1800629 LOCUS WITH DIFFERENT GENOTYPES

	GDM			Control		
	GG (n=191)	GA/AA (n=51)	p	GG (n=222)	GA/AA (n=20)	p
Age (years)	29.29±4.95	30.31±5.48	0.20	29.64±4.98	30.75±3.77	0.09
Gestational weeks	38.28±1.46	38.18±1.56	0.67	38.18±0.80	37.95±1.00	0.13
BMI before pregnancy (kg/m ²)	23.86±2.08	23.86±1.99	1.00	23.88±2.06	23.97±1.94	0.78
BMI at birth (kg/m ²)	29.80±2.20	30.44±2.28	0.07	29.66±3.03	29.72±3.65	0.90
Newborn body mass (g)	3302.27±550.07	3391.65±552.98	0.30	3283.04±429.81	3402.90±460.34	0.08
APGAR	9.80±0.52	9.78±0.58	0.81	9.78±0.44	9.80±0.41	0.77
HbA1c (%)	5.52±1.11	5.63±1.20	0.54	4.27±0.97	4.50±0.89	0.13
Zero-h plasma glucose (mM)	4.83±0.69	4.94±0.70	0.31	4.29±1.21	4.60±0.99	0.09
One-h plasma glucose (mM)	10.10±1.76	10.37±2.02	0.35	7.27±1.57	7.57±1.25	0.21
Two-h plasma glucose (mM)	8.85±1.35	8.96±1.26	0.60	6.58±1.28	6.20±1.20	0.06

(ROC) of the subjects with a diagnosis of GDM for plasma IL-6 levels was 0.8869 (95% CI: 0.8586–0.9152, $p < 0.001$, Fig. 2B). Plasma IL-10 levels in GDM patients were significantly lower than those in the control group ($p < 0.001$, Fig. 1C). The AUC of ROC for GDM diagnosis was 0.8052 (95% CI: 0.7670–0.8433, $p < 0.001$, Fig. 2D). The plasma TNF- α level in GDM patients was higher than that in the control group ($p < 0.001$, Fig. 1E). The AUC of ROC for

GDM diagnosis was 0.8908 (95% CI: 0.8618–0.9198, $p < 0.001$, Fig. 2F).

IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 and plasma IL-6, IL-10, and TNF- α levels

We further analyzed the correlation between plasma inflammatory factor levels and SNP allelic and genotypic

FIG. 2. ELISA detects the levels of IL-6, IL-10, and TNF- α in plasma of subjects. (A) Comparison of IL-6 plasma levels between GDM patients and the control group. (B) ROC of IL-6 plasma levels for a diagnosis of GDM. (C) Comparison of IL-10 plasma levels between GDM patients and the controls. (D) ROC of IL-10 plasma levels for a diagnosis of GDM. (E) Comparison of TNF- α plasma levels between GDM patients and the control group. (F) ROC of TNF- α plasma levels for a diagnosis of GDM. ELISA, enzyme-linked immunosorbent assay; ROC, receiver operating curve.

FIG. 3. Correlation between IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 and IL-6, IL-10, and TNF- α plasma levels. **(A)** Comparison of IL-6 plasma levels among GDM patients with different genotypes of the IL-6 rs1800796 locus. **(B)** Comparison of IL-6 plasma levels among subjects with different genotypes of the IL-6 rs1800796 locus in the control group. **(C)** Comparison of IL-10 plasma levels among GDM patients with different genotypes of the IL-10 rs1800896 locus. **(D)** Comparison of IL-10 plasma levels among subjects with different genotypes of the IL-10 rs1800896 locus in the control group. **(E)** Comparison of TNF- α plasma levels among GDM patients with different genotypes of the TNF- α rs1800629 locus. **(F)** Comparison of TNF- α plasma levels among control subjects with different genotypes at the rs1800629 locus.

frequencies in the subjects; these results showed that those with the IL-6 rs1800796 CG/GG genotypes had higher IL-6 plasma levels than those with the CC genotype ($p < 0.001$, $p = 0.029$; Fig. 3A, B). Persons with the IL-10 rs1800896 TT genotype had higher IL-10 levels than those with the TC/CC genotypes ($p < 0.001$, $p < 0.001$; Fig. 3C, D). Persons with the TNF- α rs1800629 GA/AA genotypes had higher TNF- α levels than those with the GG genotype ($p < 0.001$, $p = 0.001$; Fig. 3E, F).

Discussion

With the exception of trauma, most diseases are the result of a combination of genetic and environmental factors. Different genetic backgrounds can cause different populations to have different susceptibilities to certain diseases. The study of human genetic polymorphisms can help us understand the distribution of the different genotypes of various genes in different diseases.

In this study of the Han Chinese, we found that subjects with the IL-6 rs1800796 G allele had higher plasma IL-6 levels and a higher risk of GDM susceptibility than subjects with the C allele. Persons with the IL-10 rs1800896 C allele had lower IL-10 plasma levels than persons with the T allele and a higher risk of GDM susceptibility. Subjects with the TNF- α rs1800629 A allele had higher plasma TNF- α levels and a higher risk of susceptibility to GDM than subjects with the G allele.

IL-6 is an inflammatory cytokine with multiple biological functions, with a molecular weight of 26 kD. Mature IL-6 contains 184 amino acids, and the amino acid residues at positions 1–28 have no known important functions. The main role of the 97–104-amino acid region is to maintain the structural integrity of the protein. The 29–34-amino acid residue region is in close proximity to the 180–184-amino acid residue, which constitutes the IL-6 active site and plays a key role in binding to the receptor through which it exerts its biological functions (Hammacher *et al.*, 1994). The results of this study show that subjects with the IL-6 rs1800796 G allele had a higher risk of susceptibility to GDM than those who carry the C allele, and ELISA test results showed that IL-6 rs1800796 G allele carriers had higher plasma IL-6 levels, which are consistent with results of the study by Ding *et al.* (2019). The reason for this finding is likely that the –572 locus of the IL-6 promoter region affects the transcriptional efficiency of IL-6, and the G-allele at this site corresponds to a higher level of promoter activity because its cognate transcription factors bind with more affinity, which promotes increased transcription. IL-6 may cause GDM by promoting insulin resistance (Bastard *et al.*, 2002). In addition, it has been found that during development of type 2 diabetes, IL-6 can stimulate liver cells to produce acute response proteins and activate the innate immune system, thereby promoting a positive feedback cycle that further enhances the circulating levels of IL-6 and other inflammatory proteins in the body (Lindsay *et al.*, 2001), including the C-reactive protein. These two proteins can interact to affect the body's immune system and produce diabetes-related conditions such as GDM (Kesika *et al.*, 2019; Kianpour *et al.*, 2019).

In this study, we found that subjects with the IL-10 rs1800896 C allele had lower IL-10 plasma levels and higher GDM susceptibility than T allele subjects. Zhu *et al.* (2019) showed that the IL-10 rs1800896 C allele is associated with higher IL-10 levels in plasma and peripheral blood mononuclear cell culture supernatants, which is the exact opposite of the results of this study. The specific reason is unclear and may be related to the expression of IL-10 by other factors.

We found that carriers of the TNF- α rs1800629 A allele had higher TNF- α plasma levels and greater risk of GDM than carriers with the G allele. We believe that the reason may be that the A allele is more efficient in binding to transcription factors and the resulting increased expression of TNF- α is higher. TNF- α can destroy islet B cells in autoimmunity, which is a defect of B cell secretion function (Kuhl, 1998). TNF- α can also reduce insulin secretion by reducing insulin receptor autophosphorylation and promoting phosphorylation of the receptor matrix protein serine. Another possibility is that in islet β -cells, TNF- α can reduce glucose-stimulated insulin secretion (Laukkanen *et al.*, 2004). Although islet B cells secrete insulin normally because TNF- α can increase insulin resistance (Laukkanen *et al.*, 2004), to maintain stable

blood glucose levels, islet B cells secrete more insulin to compensate for lower blood glucose. Persistent hyperglycemia gradually inhibits islet β -cell secretion, which eventually leads to impaired islet β -cell function and to a gradual decline in insulin secretion. The amount of insulin cannot counterbalance insulin resistance, leading to an increase in blood glucose.

In addition, in this study, it was also found that the interactions among the IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 loci affect susceptibility to GDM, indicating that GDM is a polygenic trait.

This study is not without its limitations. First, the sample size is relatively small, thus additional studies with larger sample sizes should be conducted. Second, there is a lack of evidence from *in vitro* and *in vivo* studies supporting the correlation of IL-6 rs1800796, IL-10 rs1800896, and TNF- α rs1800629 with their respective protein expression levels.

Conclusion

In this study, we found that the IL-6 rs1800796 G, IL-10 rs1800896 C, and TNF- α rs1800629 A alleles were significantly associated with an increased risk of susceptibility to GDM.

Author Disclosure Statement

No competing financial interests exist.

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