Influence of GSTT1 and GSTP1 Polymorphisms on Type 2 Diabetes Mellitus and Diabetic Retinopathy Risk in the Chinese Population: A Case Control Study

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Research

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Abstract

Background: Studies have revealed the association of glutathione S-transferases (GSTM1 and GSTT1) deletion (null) polymorphism with the risks of developing type 2 diabetes mellitus (T2DM) and its complications. The present study aimed to investigate the relationship between GSTT1/ GSTP1 gene polymorphisms and the risks of T2DM and diabetic retinopathy (DR) in a Chinese population.

Methods: A total of 336 subjects with T2DM and a defined ophthalmologic status were recruited from the Second People's Hospital of Yunnan Province between June 2014 and October 2016. Seventy-two age-matched healthy controls were also enrolled. Physical examinations and laboratory tests were performed. The frequencies of GSTT1 and GSTP1 genotypes in all participants were determined by PCR and PCR-restriction fragment length polymorphisms (PCR-RFLP), respectively.

Results: Compared with healthy controls, the GSTT1-null genotype was significantly more common in diabetic patients with or without DR (all $P < 0.05$). However, the frequency of the GSTP1 genotype (AA, GA, GG) was comparable between the two groups. Furthermore, neither the GSTP1 nor GSTT1 genetic polymorphism was associated with the development of DR. In the present study, the risk of developing T2DM was significantly higher in subjects carrying the combined heterozygous GSTP1 (AG) and null GSTT1 genotypes (OR=0.40, 95% CI=0.21-0.74, $P=0.02$).

Conclusions: The deletion of the GSTT1 genotype was associated with a higher risk of developing T2DM, whether alone or in combination with GSTP1, indicating that the null genotype of GSTT1 may serve as a potential biomarker for T2DM in the Chinese population, which is helpful for clinicians to make more effective risk-based decisions.

Background

Type 2 diabetes mellitus (T2DM) is an endocrine metabolic disorder that is characterized by impaired insulin action in target tissues and/or insulin secretion [1]. By 2040, 642 million people are expected to suffer from T2DM [2]. Notably, long-term hyperglycemia results in many complications, such as coronary artery disease, stroke, diabetic nephropathy, peripheral diabetic neuropathy, and diabetic retinopathy (DR), all of which carry high medical and socioeconomic costs [3]. T2DM is well recognized as a multifactorial disease determined by both genetic and environmental factors [3, 4]. Although genetic studies have demonstrated that some genetic markers are associated with T2DM susceptibility and provided new insights into diabetes pathogenesis, including β -cell function, adipocytokine signaling and cell cycle regulation, the genetic basis of T2DM and the mechanisms of the observed genetic associations of T2DM still need further investigation [4, 5, 6, 7].

Oxidative stress is an important factor in the development of diabetes and its complications [8, 9, 10], and individuals with impaired antioxidant capacity were found to be at greater risk of T2DM [11, 12]. Since the retina needs more oxygen than other tissues in the body, oxidative stress may also play a pivotal role in the etiology of DR [13]. Glutathione S-transferases (GSTs), a well-known multigenic superfamily of detoxification enzymes, can effectively modulate systemic oxidative stress levels in humans [14, 15]. GSTM1 and GSTT1 are polymorphic, and the null genotypes (deletion of both alleles) are associated with reduced enzyme activity [16, 17, 18]. For GSTP1, there is a missense substitution from the A/G base at codon 105 in which isoleucine is replaced by

valine (Ile105Val), resulting in an alteration of specific enzyme activity [19]. Several GST polymorphisms, especially GSTM1, GSTT1 and GSTP1, have been intensively studied in different diseases due to their potential effects on individual susceptibility to some environmentally induced diseases [20, 21]. Recent studies have revealed the association of GSTM1/GSTT1 deletion (null) polymorphism with the risk of developing T2DM [14, 20, 22, 23, 24, 25]. Moreover, the null genotypes of GSTM1/GSTT1 were also considered risk factors for various T2DM complications, including essential arterial hypertension, neuropathy, retinopathy, nephropathy, etc. [20, 23, 26, 27]. However, few studies have focused on the relationships between GSTP1 single nucleotide polymorphism and the development of T2DM and its related complications, especially in the Chinese population. On the other hand, whether the GSTM1/GSTT1 polymorphisms are associated with risk of T2DM as well as DR is still controversial and inconsistent in the existing studies [20, 28, 29, 30].

Therefore, the present study aimed to investigate the relationships between GSTP1/GSTT1 genetic polymorphism and the risks of diabetes and DR in a Chinese population from Yunnan Province, a plateau area of Western China.

Material And Methods

Study population

In this cross-sectional study, a total of 336 subjects with T2DM and a defined ophthalmologic status were recruited from the Second People's Hospital of Yunnan Province between June 2014 and October 2016. Seventy-two age-matched healthy controls with no history of diabetes were also enrolled. The diagnosis of diabetes was in accordance with the 1999 World Health Organization criteria (fasting plasma glucose > 7.0 mmol/L, 2 h plasma glucose > 11.1 mmol/L, or both) [31]. A complete ophthalmological examination (including corrected slit-lamp microscopic examination, funduscopy examination and fundus photography) was performed by a senior ophthalmologist following a standardized protocol at the Department of Ophthalmology, the Second People's Hospital of Yunnan Province. DR was defined and staged according to the proposed Clinical International Classification Systems. The T2DM group consisted of 124 subjects with proliferative diabetic retinopathy (PDR), 70 subjects with nonproliferative diabetic retinopathy (NPDR) and 142 diabetic subjects without clinical signs of diabetic retinopathy (NDR) [32].

Ethical Principles

The study was approved by the institutional review board of the Second People's Hospital of Yunnan Province and Yunnan University and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was provided by each participant prior to the study.

Clinical Evaluation And Biochemical Assays

All participants received an interviewer-administered questionnaire to collect general information regarding age, sex and history of diabetes and underwent a basic physical examination [e.g., weight, height, waist-to-hip ratio (WHR), systolic blood pressure and diastolic blood pressure] by trained nurses. Heparinized blood samples were

collected after an overnight fast for at least 8 h. Lipid profiles [including total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL)], blood urea nitrogen (BUN), creatinine (CRE) and uric acid (UA) were measured on a Hitachi 7600 analyzer using an enzymatic assay. Glycated hemoglobin (HbA1c) was estimated by high-performance liquid chromatography using an analyzer (HLC-723 G7, Tosoh, Japan).

Dna Extraction And Gst Genotyping Analysis

DNA was extracted from the collected EDTA blood samples using a standard procedure of perchlorate/chloroform extraction [33]. Genotyping for the detection of the GSTT1 and GSTP1 genotypes was performed by PCR and PCR-restriction fragment length polymorphisms (PCR-RFLP), respectively. The albumin locus was used as an internal control to distinguish the null genotype of GSTT1 from the aborted PCRs. The primers used in the present study were as follows: GSTT1, 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' (forward) and 5'-TCA CCG GAT CAT GGC CAG CA-3' (reverse); GSTP1, 5'-ACC CCA GGG CTC TAT GGG AA-3' (forward) and 5'-TGA GGG CAC AAG AAG CCC CT-3' (reverse); and Albumin, 5'-GCC CTC TGC TAA CAA GTC CTA C-3' (forward) and 5'-GCCCTAAAAAGAAAATCGCCA ATC-3' (reverse).

Statistical analysis

Data were expressed as the mean $\pm$ standard deviation (SD) for normally distributed variables or as median (interquartile range 25–75%) for variable with a skewed distribution. For continuous variables, differences between the healthy control group and T2DM group were evaluated using Student's t-test or Mann–Whitney U test. Comparisons among diabetic groups were evaluated by one-way ANOVA or the Kruskal–Wallis test as appropriate. For categorical variables, data were presented as n (%), and comparisons between groups were analyzed by chi-square test. The relationships between GSTT1 and GSTP1 genotypes and the risks of T2DM and DR were assessed by the means of the odds ratio (OR) with 95% confidence interval (CI) limits calculated by logistic regression analysis. All tests were two-tailed, and values of $P < 0.05$ were considered statistically significant. Statistics were performed with SPSS version 13.0 (SPSS, Inc., Chicago, IL, USA).

Results

Characteristics of the study population

The demographic and clinical characteristics of T2DM patients and healthy controls are illustrated in Table 1. Compared with healthy controls, diabetic patients tended to have a longer duration of diabetes, higher BMI and WHR (all $P < 0.05$). In addition, HbA1c, total cholesterol, triglyceride, LDL-C, BUN and CRE were significantly higher in the T2DM diabetes group (all $P < 0.05$). The proportions of men and women, age, SBP and DBP were comparable between the two groups. Among the 336 T2DM patients, 142 (42.26%) had no signs of DR, 70 (20.83%) had NPDR, and 124 (36.90%) had PDR. DR was significantly associated with lower triglyceride levels but higher CRE (all $P < 0.01$).

Table 1
Characteristics of patients with T2DM with/without DR and healthy controls

	NDR	NPDR	PDR	T2DM	Control
Sample (n)	142	70	124	336	72
Age (years)	54.40 ± 14.20	53.80 ± 13.13	57.20 ± 15.43	52.20 ± 10.31	55.11 ± 11.23
Sex (male/female)	52/58	33/45	54/51	151/142	53/47
Duration of diabetes (months)	87.21 ± 55.08	104.85 ± 65.02	124.65 ± 58.11	92.65 ± 73.26*	0
BMI (kg/m ²)	24.56 ± 3.37	25.27 ± 3.65	25.02 ± 3.34	24.89 ± 3.45*	22.41 ± 2.12
WHR	0.92 ± 0.07	0.94 ± 0.07	0.93 ± 0.06	0.93 ± 0.07*	0.82 ± 0.01
SBP (mmHg)	129.51 ± 16.95	134.63 ± 19.89	142.32 ± 19.89	134.67 ± 19.36	120.21 ± 14.21
DBP (mmHg)	81.94 ± 10.35	81.99 ± 12.28	85.66 ± 10.88	83.02 ± 11.17	79.30 ± 9.24
HbA1c(%)	9.08 ± 2.45	9.75 ± 2.42	9.44 ± 2.21	9.38 ± 2.38*	5.52 ± 0.43
Total cholesterol (mmol/L)	4.79 ± 1.29	5.06 ± 1.38	5.34 ± 1.64	4.70 ± 1.21*	3.41 ± 1.21
Triglyceride (mmol/L)	3.26 ± 3.33	2.58 ± 2.41†	2.41 ± 1.45†	3.52 ± 2.32*	1.61 ± 0.32
HDL-C (mmol/L)	1.01 ± 0.65	0.98 ± 0.24	1.01 ± 0.13	1.01 ± 0.46	0.86 ± 0.25
LDL-C (mmol/L)	2.44 ± 0.92	2.67 ± 0.91	2.72 ± 0.93	2.59 ± 0.93*	2.18 ± 0.52
BUN (mmol/L)	5.27 ± 2.24	5.71 ± 2.25	7.34 ± 3.14†	5.84 ± 0.81*	4.32 ± 2.23
UA (umol/L)	349.62 ± 102.17	354.39 ± 105.94	368.02 ± 94.24	356.43 ± 102.34*	307.60 ± 26.11
CRE(umol/L)	71.64 ± 26.29	73.08 ± 38.54†	103.85 ± 76.32†	84.47 ± 55.57*	67.96 ± 6.04
Data were expressed as mean ± standard deviation (SD). *, $P \leq 0.05$ compared with normal group. †, $P \leq 0.01$ compared with NDR group.					
T2DM, type 2 diabetes mellitus; DR, diabetic retinopathy; NDR, diabetic subjects without clinical signs of diabetic retinopathy; NPDR, subjects with non-proliferative diabetic retinopathy; PDR, subjects with proliferative diabetic retinopathy; BMI, body mass index; WHR, waist to hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; CRE, creatinine.					

The Frequency Distributions Of Gstp1 In T2dm And Controls

Genotype distributions of the GSTP1 codon 105 variant in groups were in agreement with Hardy-Weinberg equilibrium (HWE) (Table 2, all $P > 0.05$). However, HWE was not suitable for the GSTT1 variants because heterozygous individuals could not be distinguished from the homozygous wild type. The distributions of

GSTP1 genetic polymorphisms among (1) patients with T2DM and healthy controls and (2) diabetic patients with and without DR are displayed in Table 3. The frequency of the GSTP1 genotypes was comparable between T2DM patients and controls, as well as diabetic patients with and without DR.

Table 2

HWE testing of genotype distributions of GSTP1 between T2DM patients with/without DR and healthy controls

		Frequencies of GSTP1 variants			χ^2	P
		AA	GA	GG		
NDR	Measured values	62	15	8	5.35	0.07
	Predicted values	56.83	25.35	2.83		
NPDR	Measured values	45	5	2	1.89	0.39
	Predicted values	43.39	8.22	0.39		
PDR	Measured values	45	10	7	5.58	0.06
	Predicted values	40.32	19.35	2.32		
Control	Measured values	47	13	2	0.33	0.85
	Predicted values	46.17	14.67	1.17		
HWE, Hardy–Weinberg equilibrium; AA, individuals carrying the homozygous AA in the GSTP1 gene; GA, individuals carrying the heterozygous GA in the GSTP1 gene; GG, individuals carrying the homozygous GG in the GSTP1 gene.						

Table 3

Genotype distributions of GSTP1 between T2DM patients with/without DR and healthy controls

Genotypes		(1)				(2)			
		Control	T2DM	P	OR (95%CI)	NDR	DR	P	OR (95%CI)
		No. (%)	No. (%)			No. (%)	No. (%)		
a.	AA	47(75)	152(76)	0.88	1.04(0.63–1.73)	62(73)	90(79)	0.32	1.29(0.78–2.12)
	GA/GG	15(25)	47(24)			23(27)	24(21)		
b.	GA	13(21)	30(15)	0.27	0.93(0.81–1.07)	15(18%)	15(13)	0.38	0.95(0.84–1.07)
	GG/AA	49(79)	169(85)			70(82)	99(87)		
c.	GG	2(3)	17(9)	0.16	1.06(0.99–1.13)	8(9)	9(8)	0.70	0.98(0.90–1.07)
	GA/AA	60(97)	182(91)			77(91)	105(92)		
DR, diabetic retinopathy; OR, odds ratio; CI, confidence interval.									

The Frequency Distributions Of Gstt1 In T2dm And Controls

For GSTT1, a significantly higher frequency of the GSTT1-null genotype was found in patients with T2DM compared with healthy controls (49.41% vs. 34.72%, $P=0.02$). Moreover, the GSTT1-null genotype was significantly more frequent in diabetic patients with or without DR than in healthy controls (all $P<0.05$, Table 4). The frequency of the GSTT1-null genotype was similar among NDR, NPDR, and PDR.

Table 4
Genotype distributions of GSTT1 between T2DM patients with/without DR and healthy controls

Group		Genotype distributions n (%)		χ^2	P	OR (95%CI)
		Null	Present			
(1)	N	25(34.72)	47(65.28)	5.13	0.02	1.84(1.08–3.12)
	T2DM	166(49.41)	170(50.60)			
(2)	N	25(34.72)	47(65.28)	4.51	0.03	1.88(1.05–3.38)
	NDR	71(50.00)	71(50.00)			
(3)	NDR	71(50.00)	71(50.00)	0.61	0.43	0.80(0.45–1.4)
	NPDR	31(44.29)	39(55.71)			
(4)	NDR	71(50.00)	71(51.00)	0.07	0.79	1.07(0.66–1.73)
	PDR	64(51.61)	60(48.39)			
(5)	NPDR	31(44.29)	39(55.71)	0.96	0.33	1.34(0.75–2.42)
	PDR	64(51.61)	60(48.39)			
(6)	NDR	71(50.00)	71(51.00)	0.04	0.85	0.96(0.62–1.48)
	DR	95(48.97)	99(51.03)			

Associations between the GSTP1/GSTT1 polymorphism and the risk of T2DM and DR

The frequency distributions of the combination of GSTP1 and GSTT1 in healthy controls and in T2DM patients with or without DR are presented in Table 5. The combination of the GSTT1-null and GSTP1 (AG) genotype conferred the highest risk of developing T2DM (OR = 0.40, 95% CI 0.21–0.74, $P=0.02$). However, the combination of the two genotypes (GSTT1 and GSTP1) showed no increased risk for developing DR in diabetic patients.

Table 5

Combination of distributions of GSTP1 and GSTT1 in T2DM patients with/without DR and healthy controls

Genotypes	(1)		(2)					
	T2DM	Control	P	OR (95%CI)	NDR	DR	P	OR (95%CI)
	No. (%)	No. (%)			No. (%)	No. (%)		
T1(+) GG	6(3.22)	1(2.00)	0.55	0.60(0.10–3.70)	5(5.88)	6(5.17)	0.85	0.95(0.55–1.65)
T1(+) AG	72(38.71)	25(50.00)	0.56	1.14(0.74–1.78)	23(27.06)	41(35.34)	0.18	1.18(0.93–1.51)
T1(+) AA	12(6.45)	7(14.00)	0.16	1.62(0.86–3.05)	5(5.88)	9(7.76)	0.58	1.13(0.75–1.71)
T1(-) GG	9(4.84)	1(2.00)	0.30	0.41(0.06–2.68)	3(3.53)	5(4.31)	0.29	1.29(0.88–1.89)
T1(-) AG	75(40.32)	10(20.00)	0.02	0.40(0.21–0.74)	39(45.88)	49(42.24)	0.68	0.95(0.75–1.21)
T1(-) AA	12(6.45)	6(12.00)	0.32	1.45(0.72–2.89)	10(11.76)	6(5.17)	0.10	0.64(0.33–1.21)
T1(+), the GSTT1 gene was present in individuals; T1(-), the GSTT1 gene was null in individuals.								

Discussion

To our knowledge, this study is the first to explore the associations of GSTP1/GSTT1 genetic polymorphism with the risks of diabetes and DR in a Chinese population. In the present study, the GSTT1 null genotype was found to be an independent risk factor for the development of T2DM, whereas no significant association with DR ($P=0.65$) was observed. GSTP1 also had no relationship with the development of T2DM and DR.

The GSTT1 gene is one of the most widely studied members of the GST family for its protective ability against oxidative stress in β -cells [34]. Currently, studies regarding the role of GSTT1 polymorphism and the risk of T2DM are inconclusive because of varying reports in different studies and in people of different ethnicities. On the one hand, some studies reported that there was no association of null GSTT1 with increasing risk for the development of T2DM [29, 34, 35, 36]. For example, although the frequency of both GSTT1-null and GSTM1-null genotypes in T2DM patients was significantly higher than that in the controls, the distribution of the GSTT1 null genotype was comparable between groups in southern Iran [29]. However, other studies reported the opposite results [14, 22, 37]. Pinheiro et al. reported that the GSTT1 polymorphism may play an important role in the pathogenesis of T2DM and complications associated with dyslipidemia in the Brazilian population [22]. Such discrepancies may result from small sample sizes, race and methodology. Considering the controversial results in this regard, a few meta-analyses were performed, and they revealed that both GSTT1 and GSTM1 conferred increased risk for T2DM [38, 39]. Our study confirmed that the frequency of null GSTT1 was higher in T2DM patients than in controls, indicating an association of the GSTT1 null genotype and the risk of T2DM in the Chinese population. This result was consistent with the only study in Chinese people conducted by Wang et al. [14]. The GSTT1-null allele has been reported to be associated with both macrovascular and microvascular

disease in T2DM patients, including diabetic nephropathy and retinopathy [20, 26, 39, 40, 41]. In recent studies, it was demonstrated that the deletion polymorphism of GSTT1 could increase the risk for diabetic retinopathy in the Caucasian population [28, 42]. In contrast, both Dadbinpour et al. and Moasser et al. reported that there was no association between GSTT1 null genotypes and retinopathy in T2DM in the Iranian population [20, 36]. In our study, we found that the GSTT1-null genotype conferred a 1.8-fold increased risk of T2DM in subjects with and without DR compared to healthy controls (all $P < 0.05$). However, the frequency of the GSTT1-null genotype was comparable between T2DM patients with and without DR, indicating that there was no significant relationship between GSTT1 null genotypes and DR in T2DM in the Chinese population. Larger prospective studies are needed to confirm our findings.

Considering that GST enzyme activity was significantly lower among individuals with the 105Val allele and that genetic polymorphisms reducing the activity of antioxidant enzymes could increase a person's susceptibility to some disease, it seems that the GSTP1 polymorphism may be associated with susceptibility to T2DM [43, 44]. However, studies regarding the association between GSTP1 polymorphism and the risk of T2DM yielded controversial results [23, 24, 25, 28, 29, 34, 42, 45, 46, 47]. Some studies suggested that the GSTP1 polymorphism was associated with susceptibility to T2DM in Caucasian and African populations [23, 24, 25, 29, 42, 46], whereas other studies did not observe a correlation between the GSTP1 polymorphism and T2DM in Caucasian and African individuals, as well as diabetes-related complications such as diabetic nephropathy in these populations [26, 34, 35, 47]. In our study, the comparison of T2DM patients with healthy controls did not reveal a significant difference in the frequency of GSTP1 genotypes, which was in accordance with previous studies. These conflicting results may be attributed to the relatively small sample size and different ethnic backgrounds of the participants. Additionally, no association was observed between the GSTP1 polymorphism and susceptibility to DR in individuals with T2DM in the present study. Thus, exploring the relationship between GSTP1 polymorphism and the risk of DR in different populations is necessary.

For the combined effect of the GSTT1 and GSTP1 genotypes and the risk of progression of T2DM and DR, a significantly higher frequency of combined GSTT1 null genotype and GSTP1 heterozygous (AG) was observed in the whole T2DM group compared with the control group (OR = 0.40, 95% CI = 0.21–0.74, $P = 0.02$) (Table 5). Our findings indicated that there may be an interaction or synergistic effect between each genotype, which may be more discriminating as risk factors for certain diseases.

Conclusions

In summary, the expression of GSTT1 was much higher in T2DM patients than in healthy controls, whereas the distribution of GSTT1 was comparable between T2DM patients with and without DR. There was no obvious association between GSTP1 polymorphisms and the risks for T2DM or DR. GSTT1 may serve as a candidate gene for T2DM in Chinese populations.

Abbreviations

T2DM

type 2 diabetes mellitus; DR:diabetic retinopathy; PCR–RFLP:PCR-restriction fragment length polymorphisms; GSTs:glutathione S-transferases; PDR:proliferative diabetic retinopathy; NPDR:nonproliferative diabetic retinopathy; NDR:diabetic subjects without clinical signs of diabetic retinopathy; WHR:waist-to-hip ratio;

HDL:high-density lipoprotein; LDL:low density lipoprotein; BUN:blood urea nitrogen; CRE:creatinine; UA:uric acid; HbA1c:glycated hemoglobin; SD:standard deviation; OR:odds ratio; CI:confidence interval; HWE:Hardy-Weinberg equilibrium.

Declarations

Ethics approval and consent to participate

The study was approved by the institutional review board of the Second People's Hospital of Yunnan Province and Yunnan University and was conducted in accordance with the principles of the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used in the present study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Yang Y, Tao WY and Li YP were responsible for the planning of the study and revising the manuscript for important intellectual content. Geng XQ and Zha L wrote the manuscript and analyzed and interpreted the data. Zhou TC, Xiong YX, Xu F, Xu B, Wang FY, Wang XL and Yang K performed the research. All authors reviewed the manuscript and approved the final draft.

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