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 Article

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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: In this case-control 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 were also enrolled. Physical examinations and laboratory tests were performed. The frequencies of GSTT1 genotypes in all participants were determined by polymerase chain reaction (PCR) followed by agarose gel electrophoresis. Genotyping of GSTP1 gene was identified by PCR amplification followed by sequencing.

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, there was no difference in the frequencies of the GSTP1 genotype (AA, GA, GG) between diabetic patients with or without DR and healthy controls. 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 (4-7).

Oxidative stress is an important factor in the development of diabetes and its complications (8, 9), and individuals with impaired antioxidant capacity were found to be at greater risk of T2DM (10, 11). 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 (12). Glutathione S-transferases (GSTs), a well-known multigenic superfamily of detoxification enzymes, can effectively modulate systemic oxidative stress levels in humans (13, 14). GSTM1 and GSTT1 are polymorphic, and the null genotypes (deletion of both alleles) are associated with reduced enzyme activity (15). 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 (16). 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 (17, 18). Recent studies have revealed the association of GSTM1/GSTT1 deletion (null) polymorphism with the risk of developing T2DM (13, 17, 19-22). Moreover, the null genotypes of GSTM1/GSTT1 were also considered risk factors for various T2DM complications, including essential arterial hypertension, neuropathy, retinopathy, nephropathy, etc. (17, 20, 23, 24). However, few studies have focused on the relationships between GSTP1 single nucleotide polymorphism (SNP) 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 (17, 25-27).

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.

Methods

Study population

In this case-control 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. The diagnosis of diabetes was in accordance with the 1999 World Health Organization (WHO) criteria (fasting plasma glucose >7.0 mmol/ L, 2h plasma glucose >11.1 mmol/L, or both) (28). A complete ophthalmological examination (including corrected slit-lamp microscopic examination, funduscopic examination and fundus photography) was performed by senior ophthalmologists blinded to the T2DM status of the participants following a standardized protocol. DR was defined and staged according to the proposed Clinical International Classification Systems (29). The T2DM group consisted of 124 subjects with proliferative diabetic retinopathy (PDR), 70 subjects with non-proliferative diabetic retinopathy (NPDR) and 142 diabetic subjects without clinical signs of diabetic retinopathy (NDR). To avoid the confounding effect of other vascular diseases, patients with malignancy, type 1 diabetes, hypertension preceding T2DM, cardiac, primary renal and liver diseases were excluded from our study. Seventy-two age-matched healthy controls were also enrolled from the physical examination center of the Second People's Hospital of Yunnan Province. In this study, the inclusion criteria for the control participants were: (1) no diabetes symptoms; (2) no history of dyslipidemia, coronary artery diseases, cerebral stroke, etc.; (3) no family history of diabetes, hypertension; (4) normal glucose tolerance assessed by a standard 75 g OGTT (fasting plasma glucose < 6.1 mmol/L, 2 h plasma glucose < 7.8 mmol/L).

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 by an auto-analyzer (Hitachi 7600, Tokyo, Japan). Glycated hemoglobin (HbA1c) was estimated by high-performance liquid chromatography (HLC-73G7, Tokyo, Japan). All reagents and kits used for testing were purchased from Invitrogen (Carlsbad, CA, USA).

DNA extraction and GST genotyping analysis

DNA was extracted from the collected EDTA blood samples using a standard procedure of perchlorate/chloroform extraction (30). The quality of DNA extraction was done using spectrophotometric measurement (Nanodrop 2000, Wilmington, USA) (31). The frequency of the GSTT1 genotypes was determined by polymerase chain reaction (PCR) followed by agarose gel electrophoresis. PCR amplification was carried out on the GeneAmp PCR system 9700 (Applied Biosystems, CA, USA). The genotyping of GSTP1 was performed by providing the PCR products to Shanghai Sangon Biological Engineering Technology &Services Co, Ltd (Shanghai, China) for sequencing. 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) (21). PCR primers were offered by Dalian Bao Biological Engineering Technology &Services Co, Ltd (Dalian, China) and reagents were obtained from Sigma-Aldrich (St. Louis, USA).

Statistical analysis

Data were expressed as the mean ± standard deviation (SD) for normally distributed variables or as median (interquartile range 25-75%) for variable with a skewed distribution. The Kolmogorov-Smirnov test was used to determine data normality. The genotype frequencies were tested for the Hardy-Weinberg equilibrium (HWE) using Pearson's chi-square test (32). 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, with post hoc Tukey test for multiple comparisons (33). For categorical variables, data were presented as n (%), and comparisons between groups were analyzed by Chi-square test or Fisher's exact test as appropriate. 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

In the present study, some cases failed genotyping for the detection of the GSTP1 genotypes (NDR, n = 57; NPDR, n = 18; PDR, n = 62; control, n = 10). Thus, a total of 199 T2DM patients with/without DR (NDR, n = 85; NPDR, n = 52; PDR, n = 62) and 62 healthy controls were included in the later analysis for GSTP1 genotypes. All participants were included in the final analysis about the associations between the GSTT1 polymorphism and the risk of T2DM and DR.

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. In addition, HbA1c, total cholesterol, triglyceride, LDL-C, BUN and CRE were significantly higher in the T2DM diabetes group (all $P < 0.05$). No significant differences were observed in the proportions of sex, age, SBP and DBP 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 $P < 0.01$).

The frequency distributions of GSTP1 in T2DM and controls

The frequency of GSTP1 polymorphism did not deviate from HWE for any of the groups (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 in the present study. 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 2. There were no differences in the frequency of the GSTP1 genotypes between T2DM patients and controls ($P = 0.88$ for AA vs. GA/GG, $P = 0.27$ for GA vs. GG/AA, $P = 0.16$ for GG vs. GA/AA), as well as diabetic patients with and without DR ($P = 0.32$ for AA vs. GA/GG, $P = 0.38$ for GA vs. GG/AA, $P = 0.70$ for GG vs. GA/AA).

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 (OR = 1.84, 95% CI = 1.08-3.12, $P = 0.02$). Moreover, the GSTT1-null genotype was significantly more frequent in diabetic patients without DR than in healthy controls (OR =

1.88, 95% CI = 1.05-3.38, *P* = 0.03, Table 3). However, we did not find statistically significant differences in the genotype distribution of GSTT1 among NDR, NPDR, and PDR.

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 4. 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.

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 was observed. GSTP1 also had no relationship with the development of T2DM and DR.

It has been documented that the antioxidant enzyme-related gene variants are involved in the pathogenesis of diabetes mellitus and its related complications in previous studies (8, 9). 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). The present study firstly 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 studies conducted on the Indian population (21), Egyptian population (35) and Japanese population (36). Considering the activity of GSTs in the detoxification of oxidative stress products and the low levels of antioxidant enzymes expressed by β-cells, deletion polymorphism of GSTT1 could contribute to susceptibility to T2DM (19), which was confirmed by the finding in our research. In vitro studies have revealed decreased expression of GSTA and GSTM in adipose tissues of obese insulin-resistant C57BL/6J mice (37). Furthermore, Liu et al have identified that activation of GST protein (A and M) expression can prevent 4-hydroperoxy-nonenal induced insulin resistance (38). These findings could support the significant association of this SNP with T2DM susceptibility identified in the present study. Therefore, measurement of GSTT1 polymorphism would appear to be valuable in the clinical management of T2DM at the early stages.

However, some studies reported different conclusions and showed that there was no association of null GSTT1 with increasing risk for the development of T2DM (26, 34, 36). In addition, although several studies have reported the association between the deletion polymorphism of GSTT1 and the risk for diabetic retinopathy in ethnic-based populations, controversial results have been identified (17, 36). In this 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. However, there was no significant difference in the frequencies of the GSTT1-null genotype between T2DM patients with and without DR, indicating that

there may be no association between GSTT1 null genotypes and DR in T2DM in the Chinese population. Such discrepancies may result from small sample sizes, ethnicity and methodology. Thus, larger prospective studies of this variant are needed to confirm our findings in the Chinese population.

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 (39, 40). But the molecular mechanisms and physiological functions of GSTs in this pathway is still unknown. It has been reported that the rs614080 SNP near GSTP1 gene was associated with higher BMI and GSTP1 expression levels in subcutaneous adipose tissue in the Mexican population, which indicating the role of GSTP1 in human obesity (41). Moreover, the deficiency of GSTP could induce glucose intolerance via JNK (its activation can trigger insulin resistance)-dependent enhancement of hepatic gluconeogenesis (42). These findings could partly provide an explanation for the association between the variant of GSTP1 genotypes and the higher risk of T2DM (20-22). However, the comparison of T2DM patients with healthy controls did not reveal a significant difference in the frequency of GSTP1 genotypes in our study, which was in accordance with previous studies (23, 34, 43, 44). Additionally, no association was observed between the GSTP1 polymorphism and susceptibility to DR in individuals with T2DM in the present study, which was consistent with the existing study (25). These conflicting results may be attributed to the relatively small sample size and different ethnic backgrounds of the participants. Gene-environment interactions may also result in the controversial findings with different populations experiencing various environment, such as pesticide use among individuals with GSTT1 and GSTM1 polymorphisms was associated with an increased risk of renal cell carcinoma (21, 45). Thus, exploring the relationship between GSTP1 polymorphism and the risks of T2DM and 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 (A/G) was observed in the whole T2DM group compared with the control group, which was consistent with previous researches (20). This result manifests the value of investigating the interaction or synergistic effect between each genotype in population studies, which may be more discriminating as risk factors for certain diseases.

It should be noted that there were several limitations. Firstly, the sample size is relatively small in this study. Further large-scale studies are warranted to confirm our findings, especially in respect of the relationship between the GSTP1 polymorphism and DR. Secondly, although the method used for GSTT1 genotype in the present study could be found in previous studies, it was not possible to distinguish homozygous and heterozygous carriers of GSTT alleles. Thus, it would be better if genotyping of GSTT1 gene was identified by sequencing in future studies.

Conclusions

In summary, the frequency of the GSTT1-null genotype was much higher in T2DM patients than in healthy controls, whereas there was no difference in the distribution of GSTT1 polymorphism between T2DM patients with and without DR. Additionally, no association was observed between GSTP1 polymorphisms and the risks for T2DM or DR. In a word, GSTT1 may serve as a candidate gene for T2DM in Chinese populations, which could help clinicians make more effective risk-based decisions and may be worth promoting in future medical research.

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

TCZ and YY were responsible for the planning of the study and revising the manuscript for important intellectual content. XQG and LZ wrote the manuscript and analyzed and interpreted the data. XYX, FX, BX, FYW, XLW, KY, WYT and YP L performed the research. All authors reviewed the manuscript and approved the final draft.

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Not applicable.

Abbreviations

T2DM: type 2 diabetes mellitus; DR: diabetic retinopathy; PCR: polymerase chain reaction; GSTs: glutathione S-transferases; PDR: proliferative diabetic retinopathy; NPDR: non-proliferative diabetic retinopathy; NDR: diabetic subjects without clinical signs of diabetic retinopathy; SNP: single nucleotide polymorphism; 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.

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Tables

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*＜0.05 compared with normal group. †, *P*＜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.

Table 2 Genotype and allele distributions of GSTP1 between T2DM patients with/without DR and healthy controls

		Genotype frequency *n* (%)						Allele frequency *n* (%)	
		AA	GA/GG	GA	GG/AA	GG	GA/AA	A	G
(1)	T2DM	152(76)	47(24)	30(15)	169(85)	17(9)	182(91)	334(84)	64(16)
	Control	47(76)	15(25)	13(21)	49(79)	2(3)	60(97)	107(86)	17(14)
	P	0.88		0.27		0.16		0.52	
	OR (95%CI)	1.04(0.63-1.73)		0.93(0.81-1.07)		1.06(0.99-1.13)		0.83(0.47-1.48)	
(2)	DR	90(79)	24(21)	15(13)	99(87)	9(8)	105(92)	195(86)	33(14)
	NDR	62(73)	23(27)	15(18)	70(82)	8(9)	77(91)	139(82)	31(18)
	P	0.32		0.38		0.70		0.31	
	OR (95%CI)	1.29(0.78-2.12)		0.95(0.84-1.07)		0.98(0.90-1.07)		1.32(0.77-2.25)	

Data were presented as number (n) and percentage (%) of observations.

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; DR, diabetic retinopathy; NDR, diabetic subjects without clinical signs of diabetic retinopathy; OR, odds ratio; CI, confidence interval.

Table 3 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)	Control	25(34.72)	47(65.28)	5.13	**0.02**	1.84(1.08-3.12)
	T2DM	166(49.41)	170(50.60)			
(2)	Control	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)			

Data were expressed as number (n) and percentage of observations.

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.

OR, odds ratio; CI, confidence interval.

Table 4 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)
	n (%)	*n* (%)			*n* (%)	*n* (%)		
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)

Data were expressed as number (n) and percentage of observations.

T1(+), the GSTT1 gene was present in individuals; T1(-), the GSTT1 gene was null in individuals. T2DM, type 2 diabetes mellitus; DR, diabetic retinopathy; NDR, diabetic subjects without clinical signs of diabetic retinopathy; OR, odds ratio; CI, confidence interval.