• Investigation •

Association of candidate gene polymorphisms with diabetic retinopathy in Chinese patients with type 2 diabetes

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Received: 2019-01-20 Accepted: 2019-08-12

Abstract

● **AIM:** To investigate the association between a set of six candidate genes and the risk of diabetic retinopathy (DR) in an urban community cohort of Chinese patients with type 2 diabetes mellitus (T2DM).

● **METHODS:** A population-based cross-sectional study. The diabetic subjects were recruited from an urban community in Beijing and categorized into groups of proliferative diabetic retinopathy (PDR), non-proliferative diabetic retinopathy (NPDR), or diabetic without any retinopathy (DWR) based on the fundus photography and duration of diabetes. Six candidate genes, including advanced glycation end product specific receptor (*AGER*), aldose reductase (*AKR1B1*), inducible nitric oxide synthase (*iNOS*), pigment epithelium derived factor (*PEDF*), tumor necrosis factor-alpha (*TNF-α*), and paraoxonase 1 (*PON1*), were chosen based on Meta-analysis of genetic association studies for DR and biochemical pathways implicated in DR progression. The allele and genotype distribution of 21 functional single-nucleotide polymorphisms (SNPs) in those 6 candidate genes were investigated using MassARRAY genotyping system.

● **RESULTS:** Among 1461 diabetic patients recruited from community, 569 were selected in following genotyping analysis, including 97 patients with PDR, 217 with NPDR, and 255 with DWR. For the promoter variant rs1051993 in *AGER* gene, the distribution of allele and genotype in PDR group differed from that in DWR group (allele: P=0.011; genotype: P=0.01). Compared with DWR, patients with PDR had lower frequencies of heterozygous genotype GT (9.8% for DWR, 1% for PDR, OR: 0.10, 95%CI: 0.01-0.72) and minor allele T (4.9% for DWR, 0.5% for PDR, OR: 0.10, 95%CI: 0.01-0.75). In multivariate model, the distribution of genotype for rs1051993 in PDR group was significantly different from that in DWR group (GT *vs* GG: OR: 0.07, 95%CI: 0.01-0.61, P<0.001). No association with DR was observed in other genotyped SNPs.

● **CONCLUSION:** The data suggest a significant association of the promoter variant rs1051993 in *AGER* gene with PDR in Chinese cohort with T2DM.

● **KEYWORDS:** polymorphisms; diabetic retinopathy; *AGER*; *AKR1B1*; *iNOS*; *PEDF*; *TNF-α*; *PON1*

DOI:10.18240/ijo.2020.02.15

Citation: Fan WY, Gu H, Yang XF, She CY, Liu XP, Liu NP. Association of candidate gene polymorphisms with diabetic retinopathy in Chinese patients with type 2 diabetes. *Int J Ophthalmol* 2020;13(2):301-308

INTRODUCTION

Diabetic retinopathy (DR), one of the most threatening microvascular complications in diabetic patients[1], leads to vision damages in approximately 75% of individuals with at least 15y of diabetes. DR is characterized by loss of pericytes, endothelial cell dysfunction, blood-retinal barrier breakdown, capillary non-perfusion, microaneurysm, hemorrhage and neovascularization[2]. Large epidemiologic studies have showed that longer duration of diabetes, poorer control of blood glucose and elevated blood pressure are the major environmental risk factors for the onset and progression of DR[3]. Despite strong evidence of DR susceptibility, these environmental risk factors do not account for the complete risk susceptibility and genetic factors were thought to be responsible for nearly 20% development of DR[4].

Attempts to identify genes in the development of DR have been conducted during the past few decades. A number of candidate genes and variants have been hypothesized to be related with DR progression[5-7]. However, the results are still inconclusive. Our previous study had evaluated the association of DR with multi-genes in Chinese patients with type 2 diabetes (T2DM), including peroxisome proliferator-activated receptor-γ (*PPARγ*), vascular endothelial growth factor-A (*VEGF-A*) and its receptor-kinase insert domain receptor (*KDR*), erythropoietin (*EPO*), aldose reductase (*AKR1B1*), protein kinase C-β (*PKC-β*), angiotensin-converting enzyme I (*ACE-I*), and intercellular adhesion molecule 1 (*ICAM-1*). However, only *VEGF-A* and *KDR* genes were observed to be associated with DR[8]. To further investigate the association of susceptible genes for DR, more candidate genes were further investigated in this current study based on our Meta-analysis or the Meta-analysis previously reported[7,9-10], including advanced glycation end product specific receptor (*AGER*), *AKR1B1*, inducible nitric oxide synthase (*iNOS*), pigment epithelium derived factor (*PEDF*), tumor necrosis factor-alpha (*TNF-α*), and paraoxonase 1 (*PON1*). The pathophysiology and potential related candidate genes for DR were shown in Figure 1.

SUBJECTS AND METHODS

Ethical Approval The study protocol was approved by the Institutional Ethics Committee of Beijing Tongren Hospital (TRECKY200907) and complies with the tenets of the Declaration of Helsinki. Written informed consents were obtained from all the participants.

Subjects and Clinical Characteristics The study cohort involved 1461 unrelated subjects with T2DM recruited from Desheng Community in urban Beijing between November 2009 and June 2012.

Diagnosis of T2DM meets the following criteria: at least two times detection of fasting plasma glucose (FPG) concentration ≥7.0 mmol/L (126 mg/dL); or a random plasma glucose concentration ≥11.1 mmol/L (200 mg/dL). In addition, individuals with a history of physician-diagnosed T2DM (self-reported) treated with anti-diabetes treatments were also enrolled. As a basic evaluation of clinical characteristics, participant's age, sex, body mass index (BMI), waist to hip ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), insulin therapy was documented and analyzed. Blood pressure was measured three measurements in a resting state, 5min apart. BMI was calculated as weight/height^2 (kg/m^2).

Methods

Ophthalmological examination All participants were subjected to a comprehensive ophthalmological examination comprising a corrected visual acuity, slit-lamp biomicroscopic examination, and dilated seven fields 30° color fundus photography (Zeiss Visucam Pro; Oberkochen, Germany).

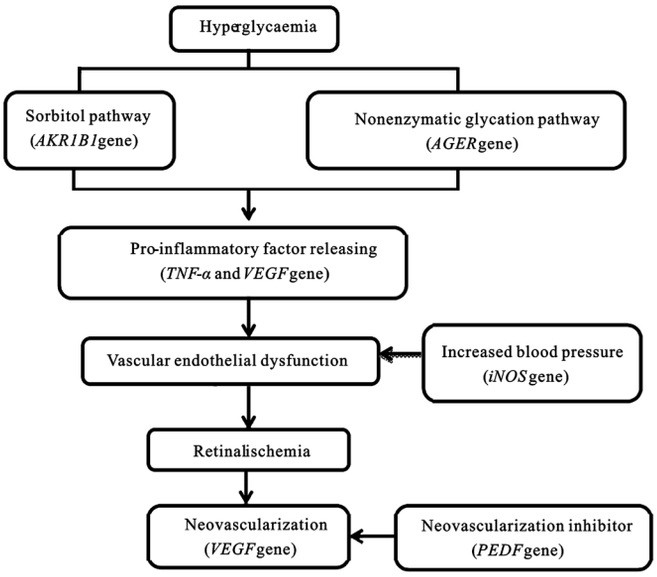

Figure 1 Pathophysiology biochemical pathways of DR.

Diabetic retinopathy grading The funduscopic findings were graded by a trained retina specialist (Yang XF). Based on the grading of retinal condition and duration of diabetes, patients were assigned into 3 groups: 1) diabetic without any retinopathy (DWR) group: patients without any retinopathy for at least 10y of diabetes or with less than 5 microaneurysms for more than 15y of diabetes; 2) non-proliferative diabetic retinopathy (NPDR) group: patients with microaneurysms and at least one of the following signs—hard exude, cotton spot, hemorrhage, intra-retinal microvascular abnormality or venous beading, but without any signs of proliferative diabetic retinopathy (PDR); 3) PDR group: patients with at least one of the following signs—neovascularization on disc or elsewhere, vitreous hemorrhage, preretinal hemorrhage or fibrovascular proliferation in at least one eye. The total DR group included both NPDR and PDR. The duration of diabetes was defined as the interval between first diagnosis of diabetes and the time of enrollment.

Biochemical Analysis Venous blood samples were collected to measure the serum levels of FPG, glycosylated hemoglobin A1c (HbA1c), creatinine, uric acid, total cholesterol, tryglycerides, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein cholesterol (LDL). Concentration of uric albuminuria ≥20 mg/L was regarded as high albuminuria.

Candidate genes and single-nucleotide polymorphisms selection Six candidate genes (*AGER*, *AKR1B1*, *iNOS*, *TNF-α*, *PON1*, and *PEDF*) that implicated in the biochemical pathways of DR progression were selected based on our Meta-analysis or the Meta-analysis previously reported[9-10]. Single-nucleotide polymorphisms (SNPs) at functional region like promoter region, 5'UTR region, or coding regions in those genes and having the minor allele frequency (MAF) more than 0.05% in Chinese ethic were included in the study (http://

Table 1 The demographic characteristics of the studied subjects

Clinical characteristics	Total DR (*n*=314)	PDR (*n*=97)	NPDR (*n*=217)	DWR (*n*=255)
Age of diabetic onset (y)	64.41±8.36[a]	65.71±7.89	63.83±8.52[a]	67.24±7.22
Gender (M/F)	138/176	40/57	98/119	111/143
Diabetes duration (y)	13.03±8.04[a]	14.45±9.18	12.40±7.41[a]	14.73±5.99
BMI (kg/m^2)	26.02±4.03[a]	25.95±3.56	26.05±4.22[a]	25.24±3.90
WHR	0.93±0.07	0.93±0.06	0.93±0.07	0.92±0.07
High albuminuria (−/+)	215/85[a]	58/38[a]	157/47[a]	217/29
SBP	138.60±16.88[a]	141.20±14.77[a]	137.40±17.66	134.20±15.86
DBP	79.03±10.22[a]	78.91±10.42	79.09±10.15[a]	76.43±10.40
Insulin therapy (yes/no)	163/150[a]	60/37[a]	103/113[a]	79/173
HbA1c (%)	7.67±1.67[a]	7.44±1.71[a]	7.78±1.64[a]	6.96±1.40
FPG (mmol/L)	9.09±3.29[a]	9.30±3.82[a]	9.00±3.03[a]	8.03±2.37
Creatinine (μmol/L)	74.97±43.91[a]	84.22±71.92[a]	70.86±21.18	68.34±16.93
Uric acid (μmol/L)	292.60±81.15	298.90±78.23	289.80±82.44	285.80±83.09
Cholesterol (mmol/L)	5.13±1.22	5.15±1.32	5.12±1.18	4.95±1.01
Triglycerides (mmol/L)	1.74±1.43	1.77±1.43	1.73±1.43	1.51±0.92
HDL cholesterol (mmol/L)	1.21±0.30	1.22±0.31	1.21±0.29	1.25±0.29
LDL cholesterol (mmol/L)	3.09±0.96	3.05±1.11	3.10±0.90	3.01±0.84

DR: Diabetic retinopathy; PDR: Proliferative diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy; DWR: Diabetes without retinopathy; BMI: Body mass index; WHR: Waist-hip ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HbA1c: Glycosylated haemoglobin; FPG: Fasting plasma glucose; HDL: High-density lipoprotein; LDL: Low-density lipoprotein. Numerical data are presented as mean±standard deviation (SD); Values of *P* (two-tailed) <0.05 was considered statistically significant; [a]*P*<0.05 compared with DWR.

www.ncbi.nlm.nih.gov/projects/SNP and http://asia.ensembl.org). When two SNPs had close linkage, only one of them was selected (r^2≥0.8, calculated by software Haploview). Eventually, 21 SNPs were selected in the study, involving 2 in 5' UTR region, 15 in promoter region, and 4 exons with missense mutation.

Genotyping Enrolled subjects were genotyped for the SNPs using SequenomMassARRAY technology (Bio Miao Biological, Beijing, China). All DNA samples passing initial quality checks were plated at a concentration of more than 20 ng/μL for processing on the platform. Genotyped SNPs with genotyping success rate <80% and deviation from Hardy-Weinberg equilibrium (HWE) in the control samples (*P*<0.001) were excluded from the following analysis.

Statistical Analysis R statistical analysis package (version 2.15.1, http://www.r-project.org) was applied to performed statistical analysis. HWE for geneotypes was tested both using online tool (http://www.genes.org.uk/software/hardy-weinberg.shtml) and genetics package in software R[8]. Unpaired Student's *t*-test (for normally distributed variables) or Wilcox test (for non-normally distributed variables) were used to explore numerical data. Categorical data were analyzed by Chi-square test. The Chi-square test was also used to analyze the distribution of alleles and genotypes. When the expected frequency was less than 5 and the results of Chi-square tests were approximately incorrect, fisher exact was used. Logistic regression was applied to calculate the odds ratio (OR) and 95% confidence interval (CI). Multivariate logistic model was used to evaluate the role of genotype and other variables. Statistical results were expressed as *P* values, OR, and 95%CI. Value of *P* (two-tailed) <0.05 was considered statistically significant for all analyses. Bonferroni correction was used in multiple testing (*P*<0.05, divided by the number of SNPs analyzed).

RESULTS

Among 1461 diabetic subjects enrolled from the Desheng Community, a total of 314 diabetic patients with DR (including 97 PDR and 217 NPDR) and 255 without any diabetic retinopathy (DWR) were selected in the study. Table 1 presents the clinical and biochemical variables of the studied diabetic individuals. Subjects in total DR group were younger than those with DWR (*P*<0.05). The duration of DM in DWR group was longer than that in DR group (*P*<0.05), and this may be ascribed to the enrollment criteria for DWR. However, when PDR was compared with DWR, the differences in age and diabetes duration were absent. Patients with DR group had higher percentage of insulin use than those in DWR group (*P*<0.05). Compared with the DWR subjects, patients with DR tended to stand higher levels of albuminuria (*P*<0.05), HbA1c (*P*<0.05), BMI (*P*<0.05), SBP (*P*<0.05), DBP (*P*<0.05), FPG (*P*<0.05) and creatinine (*P*<0.05). No statistically significant difference was found between DR and DWR with regard to sex, WHR, uric acid, cholesterol, triglycerides, HDL cholesterol and LDL cholesterol (*P*>0.05).

Table 2 Basic characteristics of candidate genes and SNPs

Gene	Position	SNP	Function	MAF	Minor allele	Sample size (DR/DWR)	HWE	
							DR	DWR
AGER	32183666	rs2070600	Exon-missense	0.19	A	314/254	>0.05	>0.05
	32184610	rs1800624	Promoter	0.16	T	314/255	>0.05	>0.05
	32185657	rs1051993	Promoter	0.04	T	314/255	>0.05	>0.05
AKR1B1	134459111	rs5053	5' UTR	0.23	C	314/254	>0.05	<0.05
	134459733	rs918825	Promoter	0.21	C	314/255	<0.001	<0.001
	134460980	rs706207	Promoter	0.26	C	305/249	<0.001	<0.05
iNOS	27769571	rs2297518	Exon-missence	0.15	A	314/254	>0.05	>0.05
	27800492	rs10459953	5' UTR	0.46	C	314/255	>0.05	>0.05
	27800806	rs2779248	Promoter	0.16	C	314/255	>0.05	>0.05
	27801555	rs2779249	Promoter	0	A	314/255		
	27782076	rs3730017	Exon-missence	0.01	T	314/255	>0.05	<0.05
	27802186	rs8078340	Promoter	0.01	A	314/255	<0.05	>0.05
TNF-α	31574699	rs1800630	Promoter	0.17	A	314/255	>0.05	>0.05
	31574531	rs1799964	Promoter	0.21	C	314/255	>0.05	>0.05
	31575254	rs1800629	Promoter	0.06	A	314/255	>0.05	>0.05
PEDF	1769982	rs1136287	Exon-missence	0.42	C	314/255	>0.05	>0.05
	1761607	rs12948385	Promoter	0	A	314/255		
PON1	95325307	rs854571	Promoter	0.32	T	314/254	>0.05	>0.05
	95325384	rs854572	Promoter	0.43	G	314/255	>0.05	>0.05
	95325909	rs705382	Promoter	0.49	C	314/254	>0.05	>0.05
	95326216	rs757158	Promoter	0.43	C	314/255	>0.05	>0.05

SNP: Single nucleotide polymorphism; MAF: Minor allele frequency; DR: Diabetic retinopathy; DWR: Diabetes without retinopathy; HWE: Hardy-Weinberg equilibrium; *AGER*: Advanced glycation end product specific receptor; *AKR1B1*: Aldose reductase; *iNOS*: Inducible nitric oxide synthase; *TNF-α*: Tumor necrosis factor-alpha; *PEDF*: Pigment epithelium derived factor; *PON1*: Paraoxonase 1.

All the selected SNPs had a successful genotype rate more than 97%. The rs12948385 in *PEDF* gene and rs2779249 in *iNOS* gene were found to be no polymorphic and had not been studied further. Besides, the rs918825 in *AKR1B1* gene had been also excluded because of its deviation from HWE in the control group (P<0.001; Table 2). The remaining 18 SNPs were further analyzed on genotype and allele distribution.

The distribution of alleles and genotypes of rs1051993 variant of *AGER* gene in PDR group differed from that in DWR group (allele: P=0.011; genotype: P=0.010) in univariate model (Table 3). Compared with DWR, patients with PDR had lower frequencies of heterozygous genotype GT (9.8% for DWR, 1% for PDR, OR: 0.10, 95%CI: 0.01-0.72) and minor allele T (4.9% for DWR, 0.5% for PDR, OR: 0.10, 95%CI: 0.01-0.75). Furthermore, a mild association with DR and NPDR was found in the exon variant rs2070600 of *AGER* gene. Compare to DWR group, the DR and NPDR both have a lower frequency of the minor allele A (OR: 0.72, 95%CI: 0.53-0.97, P=0.036; OR: 0.70, 95%CI: 0.51-0.98, P=0.046, respectively). In Table 4, after adjusting for age of diabetic onset, duration of diabetes, high albuminuria, SBP, DBP, insulin therapy, HbA1c, FPG and creatinine, the genotype distribution of rs1051993 in PDR was significantly different from that in DWR (P<0.001), and a lower frequency of GT genotype was found in PDR (GT *vs* GG: OR: 0.07, 95%CI: 0.01-0.61). This difference still remains after Bonferroni correction. However, the association of rs2070600 with DR was absent in multivariate model. No association with any type of DR was detected in other genotyped SNPs.

DISCUSSION

The present study explored the possible association of 21 polymorphisms in 6 candidate genes with DR in an urban Chinese cohort with T2DM. Significant association was observed between promoter variant rs1051993 in *AGER* gene and the risk of PDR after adjustment for possible confounding risk factors. We failed to show statistically significant association for other genes tested including *AKR1B1*, *PEDF*, *iNOS*, *TNF-α* and *PON1*.

AGER is a member of the immunoglobulin superfamily and encoded by the *AGER* gene that maps to chromosome 6p21.3. Studies on diabetic patients and animal model had shown an up-regulation of *AGER* expression and advanced glycation end products (AGEs) accumulation[11-12]. The interaction of AGEs with AGER is one of the plausible mechanisms for

Table 3 Genotype and allele frequencies of polymorphisms in DR and control groups

Gene	SNP	Genotype and allele	DWR (n=255)	Retinopathy							
				Total DR (n=314)			PDR (n=97)			NPDR (n=217)	
			n (%)	n (%)	OR (95%CI)	P	n (%)	OR (95%CI)	P	n (%)	OR (95%CI)	P
AGER	rs2070600	GG	159 (62.6)	220 (70.0)	1		67 (69.1)	1		153 (70.5)	1	
		GA	81 (31.9)	85 (27.1)	0.76 (0.53, 1.09)	0.095	27 (27.8)	0.79 (0.47, 1.33)	0.490	58 (26.7)	0.74 (0.50, 1.11)	0.120
		AA	14 (5.5)	9 (2.9)	0.46 (0.20, 1.10)		3 (3.1)	0.51 (0.14, 1.83)		6 (2.8)	0.45 (0.17, 1.19)	
		A	109 (21.5)	103 (16.4)	0.72 (0.53, 0.97)	0.036	33 (17.0)	0.75 (0.49, 1.15)	0.228	70 (16.1)	0.70 (0.51, 0.98)	0.046
	rs1800624	TT	178 (69.8)	228 (72.6)	1		67 (69.1)	1		161(74.2)	1	
		TA	68 (26.7)	75 (23.9)	0.86 (0.59, 1.26)	0.745	27 (27.8)	1.05 (0.62, 1.79)	0.972	48 (22.1)	0.78 (0.51, 1.20)	0.520
		AA	9 (3.5)	11 (3.5)	0.95 (0.39, 2.35)		3 (3.1)	0.89 (0.23, 3.37)		8 (3.7)	0.98 (0.37, 2.61)	
		A	86 (16.9)	97 (15.4)	0.90 (0.66, 1.24)	0.571	33 (17.0)	1.01 (0.65, 1.57)	1	64 (14.7)	0.85 (0.60, 1.21)	0.425
	rs1051993	GG	230 (90.2)	297 (94.6)	1		96 (99.0)	1		201 (92.6)	1	
		GT	25 (9.8)	17 (5.4)	0.53 (0.28, 1.00)	0.067	1 (1.0)	0.10 (0.01, 0.72)	0.010	16 (7.4)	0.73 (0.38, 1.41)	0.441
		TT	0	0	-		0	-		0	-	
		T	25 (4.9)	17 (2.7)	0.54 (0.29, 1.01)	0.073	1 (0.5)	0.10 (0.01, 0.75)	0.011	16 (3.7)	0.74 (0.39, 1.41)	0.452

AGER: Advanced glycation end product specific receptor; SNP: Single nucleotide polymorphism; DWR: Diabetes without retinopathy; DR: Diabetic retinopathy; PDR: Proliferative diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy; OR: Odds ratio; Values of P (two-tailed) <0.05 was considered statistically significant. OR for minor allele represents the OR for minor allele *vs* major allele.

the development of retinopathy by mediating release of pro-inflammatory molecules and free radicals that contributes to the pathology of DR[13]. To date, at least 30 polymorphisms in *AGER* gene have been reported to be associated with different vascular complications including DR. In the present study, promoter variant rs1051993 was observed to be statistically significant associated with PDR patients both in univariate and multivariate analysis. Minor allele T and heterozygous genotype GT showed a protective effect for PDR. Besides, a similar protective effect was seen in multivariate analysis for total DR patients. However, in the NPDR group, although there was a trend of decrease frequencies of genotype GT and allele T, the difference was not significant. Those results could be explained by two reasons. First, the frequency of minor allele T in SNP rs1051993 was relatively low in our cohort with a MAF less than 0.05% (MAF=0.04%). Although the sample size of PDR group was not small (n=97), low frequency of minor allele T still suggested an expand of sample size and further evaluation were needed. Second, since neovascularization only occurs in PDR but not in NPDR, and some NPDR doesn't progress into advanced PDR stage for a long time, there may be different pathogenesis accounts for different stage of DR. To our knowledge, promoter SNP rs1051993 was first time reported to have a significant association with PDR. Although the pathophysiology mechanism of polymorphism rs1051993 was not elucidated at present, we hypothesized that this variant might be implicated in the development of neovascularization and a nearby causative factor might play a directly role in the development of PDR.

In addition, a possible association was detected between SNP rs2070600, another exon variant in *AGER*, and DR in the study. The frequency of allele A was lower in individuals with DR (16.4%) compared with DWR (21.5%), which showed a significant different in univariate analysis. However, the association was absent after adjusted by confounding factors. The rs2070600 located at exon 3 and results in the amino acid substitution of glycine with serine at position 82 of the protein, which supposed to influence AGEs-AGER interaction as it occurs at a predicted N-linked glycosylation site in the same immunoglobulin variable domain as the AGE binding site[14]. The association of this variant and DR had been widely studied in different populations. However, the results are inconclusive, even in the same population. Kumaramanickavel *et al*[15] in 2002 reported a significant association of rs2070600 with DR in a Southern Indian population. The frequency of the A allele was significantly higher, 18% in the DWR group compared to 7% in the DR group. Similar result has been seen in Balasubbu's study for Indian population[16]. In contrast, a significantly higher frequency of homozygous AA genotype

Table 4 Association analysis of polymorphisms in multivariate model

Gene	SNP	Genotype	Total DR		PDR		NPDR	
			Adjusted OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
AGER	rs2070600	GG	1	0.116	1	0.540	1	0.074
		GA	0.73 (0.48, 1.13)		0.84 (0.46, 1.54)		0.69 (0.43, 1.11)	
		AA	0.41 (0.15, 1.15)		0.48 (0.11, 2.08)		0.34 (0.11, 1.09)	
	rs1800624	TT	1	0.344	1	0.696	1	0.119
		TA	0.72 (0.46, 1.14)		1.29 (0.71, 2.35)		0.59 (0.35 ,0.98)	
		AA	1.13 (0.39, 3.26)		1.28 (0.28, 5.87)		0.99 (0.32, 3.12)	
	rs1051993	GG	1	0.017	1	<0.001	1	0.214
		GT	0.41 (0.20, 0.86)		0.07 (0.01, 0.61)		0.62 (0.29, 1.32)	
		TT	-		-		-	
AKR1B1	rs5053	GG	1	0.356	1	0.283	1	0.276
		GC	0.87 (0.58, 1.30)		1.42 (0.81, 2.47)		0.77 (0.49, 1.20)	
		CC	1.82 (0.64, 5.14)		2.42 (0.59 ,9.88)		1.71 (0.54, 5.40)	
	rs918825	TT	1	0.197	1	0.169	1	0.252
		TC	0.95 (0.63, 1.41)		1.45 (0.84, 2.51)		0.84 (0.54, 1.30)	
		CC	-		-		-	
	rs706207	TT	1	0.887	1	0.540	1	0.625
		TC	0.91 (0.59, 1.40)		1.37 (0.75, 2.49)		0.80 (0.49, 1.28)	
		CC	1.03 (0.53, 2.01)		1.37 (0.54, 3.48)		1.00 (0.48, 2.09)	
iNOS	rs2297518	GG	1	0.759	1	0.988	1	0.733
		AG	1.17 (0.74, 1.85)		1.00 (0.53, 1.91)		1.22 (0.74, 2.00)	
		AA	0.87 (0.24, 3.11)		0.87 (0.15, 5.19)		0.99 (0.24, 4.07)	
	rs10459953	GG	1	0.254	1	0.140	1	0.567
		CG	1.24 (0.79, 1.93)		1.34 (0.72, 2.48)		1.22 (0.75, 2.00)	
		CC	0.81 (0.47, 1.42)		0.64 (0.28, 1.47)		0.94 (0.52, 1.73)	
	rs2779248	TT	1	0.322	1	0.325	1	0.446
		TC	1.40 (0.90, 2.18)		1.61 (0.85, 3.04)		1.36 (0.85, 2.20)	
		CC	1.20 (0.43, 3.35)		1.44 (0.39, 5.32)		1.07 (0.34, 3.41)	
	rs3730017	CC	1	0.883	1	0.679	1	0.643
		CT	1.27 (0.35, 4.68)		1.49 (0.23, 9.40)		1.64 (0.40, 6.62)	
		TT	-		-		-	
	rs8078340	GG	1	0.842	1	0.991	1	0.815
		GA	0.75 (0.23, 2.46)		0.99 (0.18, 5.40)		0.93 (0.26, 3.36)	
		AA	-		-		-	
TNF-α	rs1800630	CC	1	0.938	1	0.531	1	0.749
		CA	1.06 (0.69, 1.63)		0.90 (0.48, 1.66)		1.20 (0.75, 1.91)	
		AA	1.17 (0.36, 3.84)		2.05 (0.52, 8.03)		1.15 (0.29, 4.50)	
	rs1799964	TT	1	0.929	1	0.689	1	0.644
		TC	1.01 (0.67, 1.52)		0.83 (0.47, 1.49)		1.20 (0.77, 1.89)	
		CC	1.22 (0.44, 3.36)		1.38 (0.38, 5.02)		1.39 (0.46, 4.20)	
	rs1800629	GG	1	0.391	1	0.407	1	0.374
		GA	1.20 (0.65, 2.20)		1.43 (0.62, 3.27)		1.14 (0.58, 2.23)	
		AA	-		-		-	
PEDF	rs1136287	TT	1	0.559	1	0.756	1	0.780
		TC	0.91 (0.59, 1.39)		0.93 (0.51, 1.71)		0.98 (0.61, 1.57)	
		CC	1.22 (0.69, 2.16)		1.23 (0.58, 2.64)		1.20 (0.64, 2.25)	
PON1	rs854571	CC	1	0.899	1	0.346	1	0.619
		TC	0.96 (0.64, 1.45)		0.65 (0.36, 1.18)		1.25 (0.80, 1.96)	
		TT	1.12 (0.58, 2.16)		0.95 (0.40, 2.22)		1.08 (0.52, 2.23)	
	rs854572	CC	1	0.509	1	0.092	1	0.868
		CG	1.26 (0.81, 1.96)		2.03 (1.06, 3.91)		1.05 (0.65, 1.69)	
		GG	0.99 (0.57, 1.72)		1.67 (0.76, 3.67)		0.89 (0.48, 1.65)	
	rs705382	GG	1	0.578	1	0.132	1	0.691
		GC	1.10 (0.69, 1.76)		1.03 (0.54, 1.93)		1.18 (0.70, 1.97)	
		CC	0.85 (0.50, 1.46)		0.52 (0.24, 1.12)		0.96 (0.53, 1.72)	
	rs757158	TT	1	0.421	1	0.146	1	0.672
		TC	1.28 (0.82, 1.99)		1.88 (0.99, 3.58)		1.11 (0.69, 1.79)	
		CC	0.96 (0.55, 1.66)		1.58 (0.73, 3.44)		0.85 (0.46, 1.58)	

AGER: Advanced glycation end product specific receptor; *AKR1B1*: Aldose reductase; *iNOS*: Inducible nitric oxide synthase; *TNF-α*: Tumor necrosis factor-alpha; *PEDF*: Pigment epithelium derived factor; *PON1*: Paraoxonase 1; SNP: Single nucleotide polymorphism; DR: Diabetic retinopathy; PDR: Proliferative diabetic retinopathy; NPDR: Non-proliferative diabetic retinopathy; OR: Odds ratio; Values of *P* (two-tailed) <0.05 was considered statistically significant. OR for minor allele represents the OR for minor allele *vs* major allele.

in DR patients was detected compared with DWR (2.4% *vs* 0.64%) in another study of north Indian population[17]. While studies in Japanese[18], Malaysian population[19], found no significant association between variant rs2070600 and DR risk. For Chinese population, Yang *et al*[20] found that the frequency of the AA genotype was 12.4% in the DR group and 6.6% in the DWR group, which was opposite to our founding. And another report did not detect the association[21]. The inconsistencies of results may be explained by the difference of ethnics, demographic factors, sample size, type of diabetes, clinical features of selected patients, and enrollment criteria of control group. Furthermore, in the present study, only the patients without any sign of retinopathy for more than 10y of diabetes or with less than 5 microaneurysms for more than 15y of diabetes were selected in the control group. Therefore, the control group in our cohort was older (67.24 *vs* 62.58) and had an obvious longer duration of diabetes than that in Yang *et al*'s[20] (14.73 *vs* 5.56).

Strengths of the current study were as follow. First, the study was based on system reviews of genetic association studies for DR. All of the tested genes were selected based on our Meta-analysis or Meta-analysis previously published[9-10]. Four of the tested genes (*PEDF*, *iNOS*, *TNF-α*, and *PON1*) were first evaluated in Chinese diabetic patients. Second, we did not select SNPs from previous reported hot spots in those genes. The SNPs were picked up in the functional region with a higher MAF in Asian especially China population. Therefore, among the selected 21 SNPs, 8 polymorphisms (rs5053, rs918825, rs706207 in *AKR1B1* gene; rs1800630, rs1799964 in *TNF-α* gene; rs1800624 and rs1051993 in *AGER* gene) were first reported in DR. Finally, participants in our cohort were enrolled from a single community with well-collected information and standardized assessment of DR severity.

In conclusion, we observed an association of PDR with polymorphism rs1051993 in *AGER* gene in Chinese cohort with T2DM. We failed to show statistically significant association for other genes tested.

ACKNOWLEDGEMENTS

Foundations: Supported by the National Basic Research Program of China (973 Program) Grant (No.2007CB512201); the Beijing Municipal Health Bureau Grant (No.2009208).

Conflicts of Interest: Fan WY, None; **Gu H,** None; **Yang XF,** None; **She CY,** None; **Liu XP,** None; **Liu NP,** None.

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