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The relationship between *ACE*/*AGT* gene polymorphisms and the risk of diabetic retinopathy in Chinese patients with type 2 diabetes

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Abstract

Aims: This study aims to investigate the association between renin-angiotensin system gene polymorphism and diabetic retinopathy (DR) in Chinese patients with type 2 diabetes.

Methods: We consecutively included 1491 patients for the assessment of *ACE* I/D and *AGT* M/T gene polymorphisms in 345 DR cases and 1146 patients without retinopathy (DNR). Albuminuria was defined by urine albumin creatinine ratio and albumin excretion rate.

Results: Compared with the NDR patients, the DR cases displayed a higher proportion of diabetic nephropathy (32.68% vs. 6.52%, $\chi^2 = 150.713$, $p < 0.001$). The DR cases and DNR individuals did not differ in the frequency of genotypes and alleles of *ACE* I/D and *AGT* M/T (all $p > 0.05$). Intriguingly, DR patients with obesity showed higher frequency of DD ($\chi^2 = 4.181$, $p = 0.041$), but no significant difference exists in the other stratified BMI and hypertension analyses (all $p > 0.05$). Binary logistic regression displays that the association of the *ACE* and *AGT* gene polymorphisms in DR patients is not significant after adjusting for confounding covariates in all the comparisons.

Conclusions: The *ACE* and *AGT* gene polymorphisms are not associated with the progress of diabetes developing into retinopathy in Chinese patients with type 2 diabetes. However, more investigations are needed to further prove the association.

Keywords

T2DM, diabetic retinopathy, ACE, AGT, rennin-angiotensin system

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Introduction

Diabetic retinopathy (DR) is one of the most devastating microvascular complications of diabetes mellitus[1] and remains a major cause of visual morbidity in developed and developing countries.[2,3] Epidemiological studies have shown that DR exists in almost all individuals with long-standing type 1 diabetes mellitus (T1DM), and approximately 60% of patients with type 2 diabetes mellitus (T2DM) develop retinopathy.[4] In addition to the increased classic cardiovascular risk factors in diabetes, genetic factors may contribute to the development of these complications. Indeed, monozygotic twins with T2DM show a substantial concordance for the development of DR, suggesting that genetic factors may have a role in DR.[5] Several genetic markers have been studied,[6–10] but up to now, no main genetic locus has been identified.

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The renin-angiotensin system (RAS) consists of renin, angiotensinogen (AGT), angiotensin-converting enzyme (ACE), ACE2, angiotensin II type 1 receptor (AT1R) and AT2R.[11] In human physiology, RAS is fundamental to blood pressure regulation; as such, each component is potentially involved in the etiology of the polygenic disorder known as primary hypertension.[12] AGT is converted to angiotensin I by renin, and subsequently into angiotensin II by ACE.[13] ACE plays an important role in the regulation of systemic and renal vascular circulation by converting angiotensin I into vasoconstrictor molecule angiotensin II.[14] Higher levels of renin activity and ACE activity during the course of diabetes result in an excess of angiotensin II in the eye, abnormally constricted retinal arterioles, elevated local intravascular blood pressure, reduced retinal blood flow, increased permeability of retinal blood vessels, and ocular neovascularization.[15] Interestingly, the *ACE* gene intron 16 insertion/deletion (I/D) polymorphism accounts for about one-half of the phenotypic variance in plasma ACE levels.[16]

In recent years, several groups of researchers have focused on the relationship between RAS and DR.[17–19] However, their findings are inconsistent. Recently, we have shown an association of diabetic glomerulosclerosis with immunoreactivity of *ACE* and *AGT*.[20] Here we report that the *ACE* and *AGT* gene polymorphisms might not have a significant effect on DR in a group of Chinese T2DM patients.

Participants, materials and methods

Participants and clinical measurements

In this cross-sectional clinical-genetic association study, we consecutively recruited 1491 T2DM patients. Among them, 345 had been diagnosed as DR and 1146 were diagnosed as diabetic non-retinopathy (DNR). Cases clinically diagnosed and sampled from the database of the university-affiliated hospital before 2012 participated in this study. Patients with a controlled diet and the use of antihypertensive drugs and RAS blocking were excluded. Before taking blood samples, we informed each patient about the aim of the study and a written consent in accordance with the guidelines of the institutional review board of the Guilin Medical University was given. This study was approved by the ethical committee of Guilin Medical University (GLMC191211HL). All cases were initially diagnosed with T2DM by a qualified endocrinologist. DM was diagnosed and classified according to 1985 World Health Organization (WHO) criteria. Patients underwent detailed eye examination with ophthalmoscopy, funduscopy and fundus photography to assess DR. All patients underwent complete physical examination including body mass index (BMI), fasting plasma glucose (FPG), glycated hemoglobin (HbA_{1c}), lipid profile (triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)), albumin-to-creatinine ratio (ACR) and albumin excretion rate (AER) according the previous report.[20]

ACE *and* AGT *genotyping*

Genomic DNA of patients was extracted using protocols reported in our previous article.[21] Polymerase chain reaction (PCR) was used to determine the *ACE* gene I/D and *AGT* gene methionine (M)/threonine T polymorphisms through primers flanking the polymorphic region of intron 16 and 354 bp of exon 2, respectively. The primers used in thisstudywere5′-CTGGAGACCACTCCCATCCTTTCT-3′ and 5′-GATGTGGCCATCACATTCGTCAGAT-3′ for *ACE* I/D, and were 5′-CAGGGTGCTGTCCACACTG GACCCC-3′ and 5′-CCGTTTGTGCAGGGCCTGGCT CTCT-3′ for *AGT* M/T gene polymorphism, respectively.[20] PCR amplification revealed a 190 bp fragment (*ACE* D allele) and/or a 490 bp fragment (*ACE* I allele), and a 266 bp fragment (*AGT* M235 allele) and/or a 303 bp fragment (*AGT* 235T allele). Genotyping for the *ACE* gene I/D and *AGT* gene M/T polymorphism followed the methods described in our recent publication.[20]

Definitions and calculations

Visual acuity was assessed systematically by specialist ophthalmologists who determined the presence and graded the severity of DR according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) scale.[22] DR patients included nonproliferative and proliferative DR, and DM patients without retinopathy were defined as controls. Hypertension was defined as an average blood pressure ≥ 140/90 mm Hg on at least three different occasions at rest state or by the presence of antihypertensive treatment.[20] Renal status was defined by AER and ACR: normoalbuminuria (AER < 20 μg/min or ACR < 30 mg/g), microalbuminuria (20 ≤ AER < 200 μg/min or 30 ≤ ACR < 300 mg/g), macroalbuminuria (AER ≥ 200 μg/min or ACR ≥ 300 mg/g).[20] BMI was derived according to the following formula: BMI = body weight (in kilograms)/square of the height (in meters).[23,24] According to the BMI value, patients were divided into an obesity group (BMI ≥ 25 kg/m^2), an overweight group (23 kg/m^2 ≤ BMI < 25 kg/m^2), a normal group (18.5 kg/m^2 ≤ BMI < 23 kg/m^2) and a lean group (BMI < 18.5 kg/m^2); because of the small sample size of overweight and lean patients, we explored the distribution of genotypes and alleles only in obese and normal patients.

Statistical analysis

The data in this study were expressed as mean ± standard deviation (SD), median (interquartile range) or percentage, as appropriate. If the alleles were in Hardy-Weinberg equilibrium, the χ^2 test was performed to compare the genotype

Table 1. Clinical characteristic of 1491 patients and concordance between DR and diabetic nephropathy.

	Total	DR	DNR	*p*
n	1491	345	1146	–
Age (years)	53.42±13.60	60.08±11.34	51.42±13.59	<0.001[a]
Male (%)	603 (40.44)	148 (42.90)	455 (39.70)	0.289[b]
Smoker (%)	194 (13.01)	37 (10.72)	157 (13.70)	0.150[b]
Age at onset (years)	47.42±13.95	50.80±13.30	46.40±13.98	<0.001[a]
Duration of diabetes (years)	5.50±5.64	8.97±7.13	4.45±4.62	<0.001[a]
BMI (kg/m^2)	24.78±3.74	24.36±3.30	24.91±3.85	0.009[a]
C-peptide	1.74 (1.00–2.84)	1.92 (0.95–2.90)	1.70 (1.02–2.84)	0.737[a]
Hypertension (%)	277 (18.60)	107 (31.01)	170 (14.85)	<0.001[b]
SBP (mmHg)	133.55±22.52	145.52±24.76	129.94±20.09	<0.001[a]
DBP (mmHg)	80.15±11.41	82.94±13.34	79.31±10.62	<0.001[a]
HbA_{1c}	7.86±1.98	8.70±2.18	7.61±1.84	<0.001[a]
FPG (mmol/l)	8.82±3.53	10.10±4.36	8.43±3.14	<0.001[a]
TG (mmol/l)	1.35 (0.92–2.00)	1.44 (1.01–2.21)	1.30 (0.90–1.95)	0.002[c]
TC (mmol/l)	5.49±1.26	5.76±1.35	5.41±1.22	<0.001[a]
LDL-C (mmol/l)	3.40 (2.80–4.00)	3.60 (2.90–4.30)	3.30 (2.70–4.00)	0.982[a]
HDL-C (mmol/l)	1.21 (1.02–1.44)	1.18 (0.99–1.43)	1.21 (1.03–1.44)	0.373[a]
Plasma urea (mmol/l)	5.96±3.15	7.56±4.83	5.47±2.21	<0.001[c]
Plasma creatinine (μmol/l)	78.82±44.92	99.61±73.85	72.55±28.55	<0.001[c]
ACR (mg/g)	1.82 (0.88–6.97)	9.02 (1.79–68.58)	1.46 (0.80–3.79)	<0.001[c]
AER (μg/min)	9.35 (5.57–29.57)	50.75 (9.10–383.82)	9.11 (5.48–25.58)	<0.001[c]
Concordance between DR and diabetic nephropathy				
Diabetes without nephropathy	1209	206 (67.32)	1003 (93.48)	<0.001[b]
Diabetes with nephropathy	170	100 (32.68)	70 (6.52)	
Total	1379	306 (100)	1073 (100)	

Data are shown as means ± SD, median (interquartile range) or *n* (percentage). [a]Derived from the *t* test. [b]Derived from the χ^2 test. [c]Derived from the Mann–Whitney *U* test.

DR: diabetic retinopathy; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; Hb: hemoglobin; FPG: fasting plasma glucose; TG: triglyceride; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; ACR: albumin-to-creatinine ratio; AER: albumin excretion rate.

distribution of each polymorphism. For categorical variables, χ^2 tests were used to find out differences between groups. Differences in continuous variables were analyzed by Student's *t*-test and one-way analysis of covariance in normal distribution, and Mann–Whitney *U* test was performed for abnormal distribution. To assess the association of disease with genotype, we used binary logistic regression analysis after adjusting for various factors. The *p* value < 0.05 was defined as statistically significant. Post-hoc power calculation was conducted by using the PS software (Power and Sample Size Calculation).[25–27] Statistical analyses were performed using the SPSS program (SPSS version 15, SPSS Inc, Chicago, IL, USA).

Results

Characteristics of patient samples and clinical findings

In this study, a total of 1491 patients with T2DM were enrolled: 345 individuals with DR and 1146 diabetic patients without retinopathy. Successful genotyping for the *ACE* I/D and *AGT* M/T gene polymorphisms were obtained from 1485 patients (344 DR cases) and 1245 (293 DR cases) participants, respectively. The demographic characteristics of T2DM patients with DR are displayed in Table 1. Compared with DNR patients, the DR cases were older ($p < 0.001$) and had a longer duration of known diabetes ($p < 0.001$), a higher proportion of hypertension ($p < 0.001$), higher levels of HbA_{1c} ($p < 0.001$), FPG ($p < 0.001$), TC ($p < 0.001$), TG ($p = 0.002$), plasma urea ($p < 0.001$), plasma creatinine ($p < 0.001$), ACR ($p < 0.001$), AER ($p = 0.011$), BMI ($p = 0.009$), systolic blood pressure (SBP, $p < 0.001$) and diastolic blood pressure (DBP, $p < 0.001$).

Concordance between DR and diabetic nephropathy defined by ACR/AER

Participants were stratified according to normoalbuminuria, microalbuminuria and macroalbuminuria. According to ACR or AER, microalbuminuria and macroalbuminuria were defined as diabetic nephropathy. As shown in Table 1, the DR cases contrasted with DNR patients in having a

Table 2. Association of RAS polymorphisms with DR in type 2 diabetes.

Genotype and allele		Total	DNR	DR	Comparison	p^a	Power value
ACE	**DD**	166	124 (10.87)	42 (12.21)	DD vs. DI+II	0.489	0.112
	DI	645	507 (44.43)	138 (40.12)	DI vs. DD+II	0.157	0.291
	II	674	510 (44.70)	164 (47.67)	II vs. DD+DI	0.331	0.163
	Total	1485	1141 (100)	344 (100)	DD vs. DI vs. II	0.354	–
	D	977	755 (33.09)	222 (32.27)	D vs. I	0.689	0.261
	I	1993	1527 (66.91)	466 (67.73)			
	Total	2970	2282 (100)	688 (100)			
AGT	**MM**	30	25 (2.63)	5 (1.71)	MM vs. MT+TT	0.369	0.118
	MT	325	254 (26.68)	71 (24.23)	MT vs. MM+TT	0.404	0.128
	TT	890	673 (70.69)	217 (74.06)	TT vs. MM+MT	0.264	0.196
	Total	1245	952 (100)	293 (100)	MM vs. MT vs. TT	0.437	–
	M	385	304 (15.97)	81 (13.82)	M vs. T	0.209	0.234
	T	2105	1600 (84.03)	505 (86.18)			
	Total	2490	1904 (100)	586 (100)			

Data are shown as *n* (percentage). [a]Derived from the χ^2 test.
RAS: renin-angiotensin system; DR: diabetic retinopathy; DNR: diabetic non-retinopathy; D: deletion; I: insertion; M: methionine; T: threonine; ACE: angiotensin converting enzyme; AGT: angiotensinogen.

significantly higher proportion of diabetic nephropathy ($\chi^2 = 150.713$, $p < 0.001$), which indicated a strong concordance between DR and diabetic nephropathy.

The relation between ACE *and* AGT *gene polymorphism and DR*

The genotype and allele frequencies of the *ACE* I/D and *AGT* M/T are shown in Table 2. Genotype frequencies in all groups are all in accordance with the Hardy-Weinberg equilibrium (all $p > 0.05$). Patients with the presence of retinopathy compared to the absence had no significant association with the frequency of *ACE* genotype (DD vs. DI vs. II, $\chi^2 = 2.076$, $p = 0.354$) and *AGT* genotype (MM vs. MT vs. TT, $\chi^2 = 1.656$, $p = 0.437$), as well as the frequency of allele (D vs. I, $\chi^2 = 0.160$, $p = 0.689$; M vs. T, $\chi^2 = 1.576$, $p = 0.209$) (Table 2).

Correction for confounding risk factors

To address the confounding risk factors including BMI and hypertension, as displayed in Table 3, obese DR cases had significantly higher frequency of DD genotype ($\chi^2 = 4.181$, $p = 0.041$). In contrast, patients with normal BMI had similar RAS polymorphisms between the two groups relating to *ACE* genotype and *AGT* genotype (all $p > 0.05$), as shown in Table 4. No significant difference in the frequency of *ACE* and *AGT* genotypes and alleles relating to DR was displayed in the hypertensive and normotensive patients (all $p > 0.05$).

In binary logistic regression with groups (DNR vs. DR) as the dependent variable and age, age of onset, duration of diabetes, BMI, hypertension (%), HbA_{1c}, FPG, TG, TC, LDL-C, HDL-C, plasma urea, plasma creatinine, ACR, AER, and *ACE/AGT* genotype as covariates, the association of the *ACE* and *AGT* gene polymorphisms with DR in T2DM patients still was not significant after adjusting for confounding factors (Table 5). Otherwise, no significant association was found between *ACE* and *AGT* gene polymorphisms and DR in all the stratified BMI and hypertension analyses after adjustment for covariates (all $p > 0.05$). The power value showed that the negative findings were partly due to lack of study power displayed in Table 2 and also displayed low value in other stratified BMI and hypertension analyses (data not shown).

Discussion

In some population studies, the variants of the RAS gene have been associated with diabetes and its complications, and inhibition of RAS has prevented the risk of diabetes and its complications.[11] Most studies that evaluated the role of *ACE* gene polymorphism with DR have different claims, yet few studies that assessed the *AGT* gene polymorphism in T2DM patients with retinopathy displayed no significant association.[6,11]

In this study, we analyzed the relationship between *ACE* and *AGT* gene polymorphism and T2DM patients with retinopathy. In a total of 1491 T2DM patients, we found that no significant difference existed between DR and DNR patients regarding *ACE* or *AGT* genotype or allele, and our negative findings are consistent with previous studies.[1,11,28] These findings indicate that the suggested role of genetics in predisposition to DR is unlikely to be mediated through differences in the DNA sequence of the *ACE* or *AGT* gene, and that the I/D and M/T

Table 3. Association of RAS polymorphisms with BMI and DR in type 2 diabetes.

Genotype and allele		Patients with BMI-defined obesity					Patients with normal BMI				
		Total	DNR	DR	Comparison	p^a	Total	DNR	DR	Comparison	p^a
ACE	**DD**	69	48 (9.50)	21 (15.67)	DD vs. DI+II	0.041	96	75 (11.90)	21 (10.10)	DD vs. DI+II	0.478
	DI	278	226 (44.75)	52 (38.81)	DI vs. DD+II	0.217	364	279 (44.29)	85 (40.87)	DI vs. DD+II	0.388
	II	292	231 (45.74)	61 (45.52)	II vs. DD+DI	0.964	378	276 (43.81)	102 (49.04)	II vs. DD+DI	0.189
	Total	639	505 (100)	134 (100)	DD vs. DI VS. II	0.101	838	630 (100)	208 (100)	DD vs. DI vs. II	0.403
	D	416	322 (31.88)	94 (35.07)	D vs. I	0.321	556	429 (34.05)	127 (30.53)	D vs. I	0.310
	I	862	688 (68.12)	174 (64.93)			1120	831 (65.95)	289 (69.47)		
	Total	1,278	1010 (100)	268 (100)			1676	1260 (100)	416 (100)		
AGT	**MM**	16	14 (3.38)	2 (1.80)	MM vs. MT+TT	0.390	14	11 (2.07)	3 (1.67)	MM vs. MT+TT	0.738
	MT	133	107 (25.85)	26 (23.42)	MT vs. MM+TT	0.602	189	145 (27.26)	44 (24.44)	MT vs. MM+TT	0.460
	TT	376	293 (70.77)	83 (74.77)	TT vs. MM+MT	0.406	509	376 (70.68)	133 (73.89)	TT vs. MM+MT	0.409
	Total	525	414 (100)	111 (100)	MM vs. MT vs. TT	0.573	712	532 (100)	180 (100)	MM vs. MT vs. TT	0.703
	M	165	135 (16.30)	30 (13.51)	M vs. T	0.310	217	167 (15.70)	50 (13.89)	M vs. T	0.410
	T	885	693 (83.70)	192 (86.49)			1207	897 (84.30)	310 (86.11)		
	Total	1050	828 (100)	222 (100)			1424	1064 (100)	360 (100)		

Data are shown as *n* (percentage). [a]Derived from the χ^2 test. Obesity was defined as BMI value $\geq 25\,kg/m^2$ and normal BMI was defined as $18.5\,kg/m^2 \leq BMI < 23\,kg/m^2$.
RAS: renin-angiotensin system; BMI: body mass index; DR: diabetic retinopathy; DNR: diabetic non-retinopathy; D: deletion; I: insertion; M: methionine; T: threonine; ACE: angiotensin converting enzyme; AGT: angiotensinogen.

Table 4. Association of RAS polymorphisms with hypertension and DR in patients with type 2 diabetes.

Genotype and allele		Hypertensive patients with type 2 diabetes					Normotensive patients with type 2 diabetes				
		Total	DNR	DR	Comparison	p^a	Total	DNR	DR	Comparison	p^a
ACE	**DD**	29	20 (11.76)	9 (8.41)	DD vs. DI+II	0.375	137	104 (10.72)	33 (13.92)	DD vs. DI+II	0.164
	DI	115	68 (40.00)	47 (43.93)	DI vs. DD+II	0.519	530	439 (45.26)	91 (38.40)	DI vs. DD+II	0.056
	II	133	82 (48.24)	51 (47.66)	II vs. DD+DI	0.926	540	427 (44.02)	113 (47.68)	II vs. DD+DI	0.310
	Total	277	170 (100)	107 (100)	DD vs. DI vs. II	0.621	1207	970 (100)	237 (100)	DD vs. DI VS. II	0.115
	D	173	108 (31.76)	65 (30.37)	D vs. I	0.731	804	647 (33.35)	157 (33.12)	D vs. I	0.925
	I	381	232 (68.24)	149 (69.63)			1610	1293 (66.65)	317 (66.88)		
	Total	554	340 (100)	214 (100)			2414	1940 (100)	474 (100)		
AGT	**MM**	4	2 (1.49)	2 (2.27)	MM vs. MT+TT	0.669	26	23 (2.82)	3 (1.46)	MM vs. MT+TT	0.272
	MT	57	33 (24.63)	24 (27.27)	MT vs. MM+TT	0.659	268	221 (27.05)	47 (22.93)	MT vs. MM+TT	0.230
	TT	161	99 (73.88)	62 (70.45)	TT vs. MM+MT	0.576	728	573 (70.13)	155 (75.61)	TT vs. MM+MT	0.122
	Total	222	134 (100)	88 (100)	MM vs. MT vs. TT	0.815	1022	817 (100)	205 (100)	MM vs. MT vs. TT	0.231
	M	65	37 (13.81)	28 (15.91)	M vs. T	0.540	320	267 (16.34)	53 (12.93)	M vs. T	0.089
	T	379	231 (86.19)	148 (84.09)			1724	1367 (83.66)	357 (87.07)		
	Total	444	268 (100)	176 (100)			2044	1634 (100)	410 (100)		

Data are shown as *n* (percentage). [a]Derived from the χ^2 test.
RAS: renin-angiotensin system; DR: diabetic retinopathy; DNR: diabetic non-retinopathy; D: deletion; I: insertion; M: methionine; T: threonine; ACE: angiotensin-converting enzyme; AGT: angiotensinogen.

polymorphisms of this gene are not a useful marker to assess susceptibility to DR. Otherwise, some discrepancy existed in studies by other researchers. Cheema et al.[18] investigated the association and interaction between RAS gene polymorphisms and the development and progression of DR, which indicated RAS polymorphism was a significant risk factor both for nonproliferative DR and proliferative DR. Moreover, Nikzamir and co-authors[29] have found that the D allele of the *ACE* gene is independently associated with retinopathy in Iranian T2DM patients. Hernández et al.[30] reported the *ACE* I/D polymorphism was observed to be significantly associated with nonproliferative DR, but not with proliferative DR in a Pakistani population. Globocnik-Petrovic and colleagues[1] thought that the *ACE* I/D gene polymorphism did not contribute to the genetic susceptibility to nonproliferative, proliferative or severe proliferative DR in a group of Caucasian T2DM individuals. We estimate that the possible reason for this

Table 5. ORs of ACE/AGT genotypes for diabetic retinopathy in Chinese patients with type 2 diabetes.

		Model 1		Model 2		Model 3		Model 4	
		ORs (95% CI)	*p*	ORs (95% CI)	*p*	ORs (95% CI)	*p*	ORs (95% CI)	*p*
ACE	**DD**	0.974 (0.610-1.557)	0.914	0.999 (0.621–1.609)	0.998	0.968 (0.594–1.577)	0.897	0.649 (0.160–2.633)	0.545
	DI	0.842 (0.620-1.143)	0.269	0.839 (0.614–1.147)	0.271	0.821 (0.596–1.132)	0.230	0.986 (0.481–2.020)	0.970
	II	1	–	1	–	1	–	1	–
AGT	**MM**	0.633 (0.224-1.794)	0.390	0.632 (0.210–1.903)	0.415	0.648 (0.209–2.007)	0.452	0	0.998
	MT	0.837 (0.600-1.166)	0.292	0.837 (0.597–1.173)	0.301	0.809 (0.574–1.142)	0.228	1.220 (0.576–2.584)	0.604
	TT	1	–	1	–	1	–	1	–

Model 1 adjusted for age, sex, age onset, duration of diabetes. Model 2 adjusted for model 1 + BMI, hypertension. Model 3 adjusted for model 2 + HbA1c, FPG, TG and TC. Model 4 adjusted for model 3 + plasma urea, plasma creatinine, ACR and AER.
ORs: odds ratios; ACE: angiotensin-converting enzyme; AGT: angiotensinogen; D: deletion; I: insertion; M: methionine; T: threonine; BMI: body mass index; Hb: hemoglobin; CI: confidence interval; FPG: fasting plasma glucose; TG: triglyceride; TC: total cholesterol; AER: albumin excretion rate.

discrepancy in these results may be related to ethnic differences and lifestyle factors. A meta-analysis of larger numbers of patients regarding *ACE* I/D polymorphism on risk of DR was performed and found that *ACE* I/D gene polymorphism might contribute to DR development, especially in the Asian T2DM group.[31] We speculate that the high heterogeneity in these results may lead to different outcomes in this study. Another meta-analysis was conducted to assess the relationship between the pattern of *ACE* gene polymorphism and T2DM patients' presence or absence of retinopathy and found that the frequency of the DD genotype was not significantly different between the groups.[32] Abhary et al. also conducted a meta-analysis and found no significant association between *ACE* polymorphisms and DR.[33] The finding was consistent with our observations in this study.

In our study, we further assessed the *ACE* and *AGT* gene polymorphism with DR relating to obese and hypertensive patients. We have found that patients with normal BMI had similar RAS polymorphisms between the two groups relating to *ACE* genotype and *AGT* genotype, but in obese individuals, DR cases had a significantly higher frequency of DD genotype. Previous work reported that significant differences in FPG,[34] DM duration,[29] age, BMI, SBP, DBP[6] and ACE activity[29,34] existed between the DNR and DR groups. T2DM coexists with immunological disturbances[35–37] and could lead to retinopathy.[38,39] Therefore, clinical characteristics of T2DM patients such as obesity may interact with genetic factors for the development of retinopathy, as highlighted in a previous report.[12] However, Pan et al.[40] found that frequencies of the *ACE* genotypes (DD, ID and II) were not significant among the BMI-defined groups of Chinese patients with T2DM. In our study, regarding the outcome of binary logistic regression on the stratified BMI analyses after adjustment for covariates, no significant association between *ACE* and *AGT* gene polymorphisms and the risk of retinopathy in diabetes patients was found in all the comparisons, and the possible reasons were due to the small sample or others. Therefore, more attention should be paid to whether the *ACE* and *AGT* gene polymorphisms with the interaction of BMI facilitated the development of diabetes to DR.

Moreover, this study disclosed that no significant difference was displayed with respect to the frequency of *ACE* and *AGT* genotypes and alleles relating to the risk of DR in hypertensive and normotensive T2DM patients, suggesting that potential interactions of the RAS gene polymorphisms with blood pressure did not promote the pathogenesis of DR. Thomas et al.[12] found no significant relationship was identified between these polymorphisms and blood pressure in a Chinese population relating to *ACE* and *AGT* genotypes. Zarouk and colleagues[41] found that the DD genotype and the D allele of the *ACE* gene were associated with hypertension and T2DM in Egyptian patients. Ramachandran et al.[42] discovered that the D allele of the *ACE* gene was associated with essential hypertension and T2DM in Malaysian individuals. Nakhjavani et al.[43] concluded that the DD polymorphism in the *ACE* gene was independently associated with hypertension in Iranian type 2 diabetic patients. Xue and colleagues[44] showed that the M allele of the *AGT* gene was probably related to hypertension in Chinese female T2DM patients. Zhou et al.[45] predicted that *ACE* gene deletion is a risk factor for hypertension but is not a risk factor for diabetes in an elderly population. RAS is clearly involved in the maintenance of blood pressure[46] and a significant relationship exists between blood pressure and retinopathy.[47] Taken together, no significant difference in the *ACE/AGT* polymorphisms existed after adjusting for hypertension. The possible reasons may be due to the different ethnic groups, the diabetic control group with potential complications, small sample size or lower power value in some comparisons.

Some limitations should be noticed when interpreting our findings. Firstly, because of lack sufficient data, we were unable to perform further analysis of the relationship between RAS gene polymorphism and DR according to glycemic index, TG, HbA_{1c}, duration of DM and so on. Secondly, the *ACE2, AT1R* and *AT2R* gene polymorphisms of the RAS system not further analyzed might play an

important role in the relationship, which may influence our findings. Otherwise, the lower power values may also have influenced the findings. Even so, we hope that our findings may provide a line of evidence for further studies.

Conclusions

In summary, the results in this study indicate no significant association between *ACE/AGT* gene polymorphisms and DR in Chinese patients with T2DM; however, more investigations are needed to further prove our findings.

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