ORIGINAL ARTICLE

Association of polymorphisms in the *RAGE* gene with serum CRP levels and coronary artery disease in the Chinese Han population

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The role of an advanced glycation end product/receptor for advanced glycation end product (AGE/RAGE) system in the pathogenesis of coronary artery disease (CAD) is not fully understood. To clarify whether polymorphisms of the *RAGE* gene were related to CAD, we performed a case–control study in Chinese Han patients. The allele frequencies and genotype distribution combinations of the −429T/C, 1704G/T and G82S polymorphisms of the *RAGE* gene were compared in 200 cases of hypertension (HT), 155 cases of CAD combined with HT (CAD&HT), 175 cases of CAD and 170 control subjects. Polymerase chain reaction-restriction fragment length polymorphism was used for detection of genotypic variants. The S allele frequency of the G82S polymorphism was higher in the CAD (odds ratio (OR), 2.303, 95% confidence interval (CI) 1.553–3.416; $P<0.001$, $P_{corr}<0.003$) and CAD&HT (OR, 1.842; 95% CI 1.219–2.785; $P<0.003$, $P_{corr}<0.009$) groups when compared with the control group. However, the S allele frequency was not significantly different between the CAD and the CAD&HT patient groups ($P=0.223$), and no statistically significant difference of genotype or allele frequency distributions was observed in the HT group ($P>0.05$). Meanwhile, serum CRP was significantly associated with the G82S variant. Haplotype-based logistic regression analysis revealed that haplotype G-Ser-T (OR, 1.670; 95% CI, 1.017–2.740; $P=0.043$), compared with the reference haplotype T-Gly-T, was associated with an increased risk of CAD after adjusting for other risk factors. Further analysis limited to non-diabetic participants exhibited similar significant findings. The haplotype carrying the G82S variant of the *RAGE* gene was significantly associated with an increased risk of CAD, but not with HT patients. Moreover, a remarkable association of the G82S variant with serum CRP levels implied that the prevalence of *RAGE* 82S allelic variation might influence susceptibility to CAD by affecting vascular inflammation.

INTRODUCTION

Recently, an important role of receptor for advanced glycation end product (RAGE) in the pathogenesis of vascular diseases has generated great interest. RAGE is a multiligand member of the immunoglobulin superfamily of cell surface molecules and mediates vascular inflammation through interaction with distinct proinflammatory ligands such as advanced glycation end products (AGEs), members of the S100/calgranulin superfamily, amphoterin and amyloid-β protein.[1] Sustained RAGE–ligand interaction in multiple settings triggers rapid generation of reactive oxygen species (ROS), amplifies immune/inflammatory reactions, perturbs endothelium properties, upregulates irreversible formation of crosslinked collagen and induces cardiac myocyte apoptosis, thereby imparting a potential impact on the cardiovascular system.[1,2] Meanwhile, an upregulated expression of RAGE in human atherosclerotic plaques suggests an important role of

RAGE gene variation in the pathogenesis of atherosclerosis and coronary artery disease (CAD).[3]

The gene encoding for RAGE (LocusID 177; chromosome 6p21.3) in the major histocompatibility complex, a region of the genome containing a number of inflammatory genes, contains 11 exons and a 1.7-kb 5′-flanking region.[4] Several variants of the *RAGE* gene, including functional polymorphisms −429T/C (database for single-nucleotide polymorphism (SNP) rs1800625) in the promoter region, 1704G/T (database for SNP rs184003) in intron 7 and G82S (database for SNP rs2070600) in exon 3, have been implicated in the development of diabetes-associated atherothrombotic disorders. For example, a recent study suggested that the HLA 8.1 ancestral haplotype was strongly linked to the C allele of the −429T>C polymorphism of the *RAGE* gene.[5] Experimental studies elucidated that cells expressing the *RAGE* G82S polymorphism showed enhanced ligand-binding affinity

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and led to increased ligand-stimulated activation of proinflammatory mediators compared with the common *RAGE* G82 isoform.[6] Furthermore, an association of the G82S polymorphism with diabetic retinopathy has been reported recently and the G–A haplotype containing 1704G and 82S alleles was suggested to be a risk marker of diabetic retinopathy in Chinese type 2 diabetic patients.[7] Interestingly, it is worth noting that non-diabetic/non-obese persons with homozygosity for the minor S allele (S/S) of the G82S polymorphism had elevated risk factors for cardiovascular disease, including a low soluble form of RAGE levels, inflammation, oxidative stress and insulin resistance, compared with those bearing at least one G allele.[8] All of these might indicate a possible genetic contribution of functional variants in the *RAGE* gene to the development of atherosclerosis and CAD.

C-reactive protein (CRP), first described as an inflammatory biomarker, is known to be a strong independent predictor of future coronary events in healthy subjects.[9] Recent *in vitro* and *in vivo* studies have shown that CRP stimulates cell adhesion molecules, chemokines, endothelin-1 release from endothelial cells, upregulation of RAGE and E-selectin expression, and enhancement of monocyte–endothelial cell adhesion.[10] In the vessel wall, CRP promotes the migration and proliferation of vascular smooth muscle cells and amplifies the production of ROS.[11] These observations argue strongly in favor of a specific proatherosclerotic effect of CRP, in that CRP functions not only as a biomarker for the risk of cardiovascular disease but also as an active mediator of atherosclerosis. Therefore, functional variants of the *RAGE* gene may be associated with elevated CRP levels during the progression of atherosclerotic events.

However, to date, few gene association studies have been conducted to assess the role of common *RAGE* gene polymorphisms (−429T/C, 1704G/T and G82S) in the pathogenesis of CAD in the Chinese Han population. We anticipate that our investigation will lead to a better understanding of the influence of these genetic variants, or haplotypes thereof, on cardiovascular disease, especially on CAD and hypertension (CAD&HT) in Chinese Han patients.

MATERIALS AND METHODS

Subjects
This study randomly enrolled 200 (99/101, male/female) unrelated HT cases, 175 (133/42, male/female) unrelated CAD cases, 155 (107/48, male/female) unrelated HT combined with CAD cases and 170 age-matched (72/98, male/female) unrelated healthy controls with no history of vascular disease. The study was conducted from July 2004 to December 2005 and enrolled individuals from the city of Guangzhou in the GuangDong province. The average age was 60.6 ± 12.9 years for HT patients, 60.8 ± 11.2 years for CAD patients, 63.5 ± 11.0 years for CAD&HT patients and 61.0 ± 10.8 years for healthy controls. HT patients did not use antihypertensive medication at the beginning of the study and none of the controls had a family history of diabetes or cardiovascular disease. Ethnic information and history of cardiovascular risk factors, such as HT, diabetes or hyperlipidemia, were recorded through patient self-reporting. In addition, one requirement was that all four grandparents and both parents of each patient be of the same ethnic group as the subject. Blood pressure was measured after 10 min of rest in a sitting position. Systolic blood pressure and diastolic blood pressure values were calculated as the mean of three consecutive physician-obtained measurements. The diagnosis of HT was defined as systolic blood pressure $\geqslant 140$ mm Hg and diastolic blood pressure $\geqslant 90$ mm Hg according to the World Health Organization criteria; CAD was diagnosed as the presence of unstable angina, acute myocardial infarction or old myocardial infarction on the basis of the diagnostic changes of either cardiac enzymes or electrocardiograms, or through coronary angiography defined as $\geqslant 50\%$ luminal stenosis of vessels greater than 1.5 mm in diameter. All subjects with secondary HT, renal failure, liver disease, tumor and autoimmune diseases were excluded from this study. Ethics approval for conducting

this study was granted by the local bioethical committee and informed consent was obtained from each participant beforehand.

Biochemical analysis
Serum concentrations of CRP, fasting glucose, triglycerides, total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were measured using the methods from the Department of Clinical Laboratory in Nanfang Hospital, in affiliation with the Southern Medical University.

Cardiac ultrasonography
Two-dimensional and Doppler echocardiography were performed using a Color Doppler Ultrasound system (ACUSON SEQUOIA 512, Sequoia, Siemens Medical Systems, Mountain View, CA, USA), with a probe frequency of 3.5 MHz. Left ventricular ejection fraction, left ventricular internal diameter at end-diastole, left ventricular internal diameter at end-systole and conventional Doppler diastolic parameters (*E/A* the peak mitral flow velocity of the early rapid filling wave (E), peak velocity of the late filling wave caused by atrial contraction (A)) were measured in all subjects by the Ultrasound Department in Nanfang Hospital, in affiliation with the Southern Medical University.

Genotyping
On the basis of the literature, three SNPs of the *RAGE* gene (−429T/C in the promoter region, 1704G/T in intron 7 and G82S polymorphism in exon 3) were selected for this study, as all three *RAGE* polymorphisms may contribute to the development of atherosclerosis or be involved in the pathogenesis of CAD.

Genomic DNA was extracted from peripheral venous blood leukocytes using a commercial blood DNA extraction kit (Genomic DNA purification kit; TaKaRa Biotechnology, Dalian, China) and was stored at $-20\,^{\circ}$C until use for genotype testing. (Table 1). *Alu*I was the specific restriction endonuclease used for −429T/C and G82S, while *Bfa*I was the endonuclease used for 1704G/T (Takara Company).

RAGE gene DNA sequences were amplified by PCR in a 25 μl reaction volume containing 1 μl of forward and reverse primers each (Table 1), 4.0 μl of template DNA, 2.0 μl of deoxynucleotide triphosphates (dNTPs), 0.25 μl of *Taq* DNA polymerase (Takara Company) and 2.5 μl of 10×PCR buffer. Amplification was performed using the Gene Amp PCR system 9600 (Perkin-Elmer, Waltham, MA, USA), with an initial denaturation of 20 min at 95 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, primer extension at 72 °C for 45 s, followed by a final extension for 10 min at 72 °C.

After confirmation by 1.5% agarose gel electrophoresis, PCR products were digested in a 20 μl reaction volume containing 1 μl of restriction endonucleases (*Alu*I for −429T/C or G82S, and *Bfa*I for 1704G/T; Takara Company) at 37 °C for 12 h, and the digestion products were electrophoresed on a 2% agarose gel for 1 h, visualized under ultraviolet light and the images captured by a charge-coupled device camera (UVIPhoto-V99, UVIBand-V99, UVI; SJ, Cambridge, UK). Genotypic outcomes were confirmed by two independent observers who were unaware of the phenotypes. Conflicting results were settled by a joint reading and, when necessary, by a repeat genotyping.

Statistical analysis
Values were expressed as mean ± s.d. unless otherwise stated. All statistical analyses were conducted using SPSS software version 13.0 (SPSS, Chicago, IL, USA). Differences in allele frequencies of each SNP among cases and controls were compared using the χ^2-test and assessed as an odds ratio (OR) of the minor allele with the corresponding 95% confidence interval (CI) and *P*-value, using the major allele as a reference. Tests for Hardy–Weinberg equilibrium were conducted using χ^2-tests. Differences in biochemical and echocardiographic data among research/genotype groups were assessed by one-way analysis of variance. Spearman's correlation analysis for adjustment of age, gender, smoking status, alcohol use, medical treatment assignment and diabetes was also performed among variables. Considering the fact that the number of homozygous subjects with a minor allele was <5%, associations between genotypes (major/heterozygous+minor) and research groups were calculated by multivariate logistic regression analyses, with atherosclerotic risk factors as covariates. Atherosclerotic risk factors adjusted in the model included age,

gender, blood pressure, smoking status, alcohol use, medical treatment assignment, glucose, serum lipid profiles (triglycerides, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol), ejection fraction, left ventricular internal diameter at end-diastole, left ventricular internal diameter at end-systole, *E/A* and diabetes. Haplotype analysis was performed with PHASE v2.1.1. The relationship between haplotypes and research groups was examined using a haplotype-based logistic regression analysis with baseline parameterization. In addition, prespecified analysis was also performed and was limited to non-diabetic participants. In assessing the associations between *RAGE* polymorphisms/haplotypes and HT or CAD, the Bonferroni correction was adopted for replication study to avoid false positives. P_{corr} indicated that the P-value was corrected by Bonferroni correction. A two-tailed P-value of less than 0.05 was considered significant.

RESULTS

The main baseline clinical characteristics of the four study groups (HT, CAD&HT, CAD and CTRL groups) are summarized in Table 2. As expected in this cohort study, research subjects had a higher prevalence of traditional atherosclerotic risk factors at baseline compared with control subjects. Systolic blood pressure, diastolic blood pressure, CRP, triglycerides, total cholesterol, low-density lipoprotein

Table 1 Primers used for PCR assays in the *RAGE* gene

Primer	RAGE *gene*	Primer sequences (from 5' to 3')	Position in RAGE	T_a (°C)	Product (bp)
−429T/C	Promoter	1 CTCCTCACTTGTAAACTTGT	−579 to −560	55	243
		2 ATAGGGTTCAGGCCAGACT	−355 to −337		
1704G/T	Intron 7	1 GGAGCCAGAAGGTGGAGCAGTAG	8223–8245	55	425
		2 GTCTCACCGATGATGCTGATGATG	8627–8647		
G82S	Exon 3	1 GTAAGCGGGGCTCCTGTTGCA	7007–7027	55	397
		2 GGCCAAGGCTGGGGTTGAAGG	7383–7403		

Abbreviation: RAGE, receptor for advanced glycation end product.

Table 2 Clinical characteristics of study participants

	HT (n=200)	CAD&HT (n=155)	CAD (n=175)	CTRL (n=170)	F/χ^2-value	P-value
Age (years)	60.6±12.9	63.5±11.0	60.8±11.2	61.0±10.8	2.301	0.076
Male (%)	49.5	69.0	74.3	42.4	49.887	<0.001
Smoking status (%)					43.028	<0.001
Never	77.0	62.6	66.3	85.3		
Past	13.0	30.3	22.9	5.3		
Current	10.0	7.1	10.9	9.4		
Alcohol use (%)					11.682	0.069
Rarely/never	90.5	84.5	89.1	90.6		
Past	6.5	13.5	9.7	5.3		
Current	3.0	1.9	1.1	4.1		
SBP (mm Hg)	156.0±28.8*,**,***	146.2±26.0*,**,†	120.0±22.3***,†	118.3±20.3***,†	100.290	<0.001
DBP (mm Hg)	88.2±17.9*,**,***	82.4±16.4*,**,†	72.7±13.3***,†	71.7±14.2***,†	43.754	<0.001
CRP (mg l⁻¹)	5.8±2.2*,**,***	7.4±2.8*,†	7.7±3.2*,†	3.8±2.1**,***,†	88.244	<0.001
TG (mmol l⁻¹)	1.9±1.4*	2.1±1.6*	1.7±1.2	1.4±0.9***,†	9.497	<0.001
TC (mmol l⁻¹)	5.0±1.1*,**	4.8±1.1	4.7±1.2†	4.6±1.0†	4.752	0.003
HDL-C (mmol l⁻¹)	1.2±0.3**	1.2±0.3*	1.2±0.3*,†	1.3±0.3**,***	4.620	0.003
LDL-C (mmol l⁻¹)	2.8±0.8*,**,***	2.7±0.8†	2.6±0.8†	2.5±0.7†	5.039	0.002
FG (mmol l⁻¹)	6.0±2.2*	5.8±1.5*	6.2±5.2	5.1±1.0***,†	14.215	<0.001
LVEF (%)	61.0±10.0**	58.1±11.5**	52.8±15.2*,***,†	59.4±10.8**	9.798	<0.001
E/A	0.9±0.5*,**	1.0±0.7*,**	1.1±0.5***,†	1.2±0.7***,†	16.546	<0.001
LVIDd (mm)	45.4±8.4**	47.2±7.5	49.0±8.9†	48.1±10.7	4.876	0.002
LVIDs (mm)	26.5±9.3**	28.9±10.0**	32.2±11.3***,†	29.8±11.9	8.199	<0.001
History of diabetes (%)	37.0	38.1	21.1	0	89.055	<0.001
Aspirin use (%)	46.5	74.2	63.4	14.7	134.433	<0.001
ACEI/ARB use (%)	67.0	68.4	41.7	17.6	119.831	<0.001
β-Blocker treatment (%)	34.5	52.9	42.3	11.8	66.286	<0.001
Statin treatment (%)	40.0	64.5	58.3	12.9	109.051	<0.001

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ANOVA, analysis of variance; ARB, angiotensin α receptor blocker; CAD, coronary artery disease; CAD&HT, coronary artery disease combined with hypertension; CRP, C-reactive protein; DBP, diastolic blood pressure; *E/A*, conventional Doppler diastolic parameters; FG, fasting glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diameter at end-diastole; LVIDs, left ventricular internal diameter at end-systole; RAGE, receptor for advanced glycation end product; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.
Values are expressed as mean values ± s.d., unless otherwise stated. One-way ANOVA was performed for continuous variables and χ^2-test for categorical variables.
*P<0.05 vs the control group; **P<0.05 vs the CAD group; ***P<0.05 vs the CAD&HT group; †P<0.05 vs the HT group.

Table 3 SNP genotype frequencies in HT, CAD&HT, CAD and CTRL groups

Subjects	CTRL n=170	HT n=200	CAD&HT n=155	CAD n=175	CTRL vs HT χ²P	P-value	Pcorr	CTRL vs CAD&HT χ²P	P-value	Pcorr	CTRL vs CAD χ²P	P-value	Pcorr	CTRL vs HT+CAD&HT χ²P	P-value	Pcorr	CTRL vs CAD+CAD&HT χ²P	P-value	Pcorr	CAD&HT vs CAD χ²P	P-value	Pcorr
refSNP ID Genotype																						
rs2070600 (G82S)																						
GG	74.7%	70.0%	59.4%	52.0%																		
GS	24.1%	27.5%	37.4%	44.0%																		
SS	1.2%	2.5%	3.2%	4.0%	0.463	0.090	0.270	0.010	0.019	0.057	<0.001	<0.001	<0.003	0.076	0.108	0.324	<0.001	0.001	0.003	0.244	0.113	0.339
rs1800625 (−429T>C)																						
TT	83.2%	79.0%	74.1%	78.9%																		
TC	16.0%	21.0%	25.9%	21.1%																		
CC	0.8%	0	0	0	0.338	0.946		0.250	0.559		0.568	0.538		0.180	0.880		0.237	0.153		0.545	0.599	
rs184003 (1704G/T)																						
GG	65.5%	70.3%	68.5%	61.4%																		
GT	31.9%	27.5%	25.9%	29.8%																		
TT	2.5%	2.2%	5.6%	8.8%	0.718	0.797		0.477	0.696		0.176	0.636		0.643	0.828		0.229	0.972		0.682	0.445	

Abbreviations: CAD, coronary artery disease; CAD&HT, coronary artery disease combined with hypertension; CTRL, control; $\chi^2 P$, chi-squared P-value; *E/A*, conventional Doppler diastolic parameters; FG, fasting glucose; HDL-C, high-density lipoprotein cholesterol; HT, hypertension; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diameter at end-diastole; LVIDs, left ventricular internal diameter at end-systole; P_{corr}, P-value after Bonferroni correction; SNP, single-nucleotide polymorphism; TC, total cholesterol; TG, triglyceride.
P-values were calculated by multivariate logistic regression analysis between genotypes (major/heterozygous+minor) and research groups with atherosclerosis risk factors as covariates, and was <5% when considering the number of homozygous subjects with a minor allele. Atherosclerotic risk factors adjusted in the model included age, gender, blood pressure, smoking status, alcohol use, medical treatment assignment, FG, serum lipid profiles (TG, TC, HDL-C, LDL-C), EF, LVIDd, LVIDs, E/A and diabetes.

and fasting glucose were significantly higher, whereas *E/A* was significantly lower in the HT group when compared with the CTRL group. In the CAD&HT group, systolic blood pressure, diastolic blood pressure, CRP, triglycerides and fasting glucose were higher, whereas high-density lipoprotein and *E/A* were lower compared with control subjects. On comparing the CAD group with control patients, it was found that CRP was significantly higher, whereas left ventricular ejection fraction and high-density lipoprotein were significantly lower. Comparison of other risk factors between research groups and the CTRL group revealed no significant difference.

The association of *RAGE* SNP genotypes with HT, CHD&HT and CHD patients is shown in Table 3. The observed genotypic distributions were in Hardy–Weinberg equilibrium in the entire study. Interestingly, the frequency of the mutated genotypes (GS&SS) and the S allele frequency of the G82S polymorphism in the Chinese Han population (GS&SS, 25.3%; S, 13.2%) were similar to those in Asian Koreans (GS&SS, 30.2%; S, 16.0%; $P>0.05$), but statistically higher when compared with the Caucasian-American population (GS&SS, 8.0%; S, 3.95%; $P<0.01$). Meanwhile, the G82S polymorphism showed an association with CAD, as 26.0% of CAD patients were carriers of the S allele vs 13.2% in the CTRL group (OR, 2.303; 95% CI, 1.553–3.416; $P<0.001$, $P_{corr}<0.003$). However, the S allele frequency was not significantly different between patients in the CAD (26.0%) and CAD&HT (21.9%) groups (OR, 1.250; 95% CI, 0.873–1.792; $P=0.223$). Considering that CAD is a subtype of CAD&HT, we compared the S allele frequency of G82S in the CTRL group with that in the CAD+CAD&HT groups, and found that G82S was significantly associated with CAD&HT patients as 24.1% of CAD+CAD&HT patients are carriers vs only 13.2% in the CTRL group (OR, 2.081; 95% CI, 1.450–2.985; $P<0.001$, $P_{corr}<0.003$). In addition, a stratified χ^2-test showed that mutated genotypes (GS&SS) were associated with male CAD patients ($P<0.01$, data not shown). Furthermore, logistic analyses adjustment for atherosclerotic risk factors revealed an asso-

ciation of the G82S polymorphism with an increased risk of CAD and CAD&HT ($P<0.001$ and $P=0.019$, respectively), whereas this was not the case for HT patients ($P=0.090$). No significant differences in allele distributions were observed between the other two SNPs and research groups, indicating that the other two SNPs were not significantly associated with CAD or HT ($P>0.05$).

The differences in haplotype distributions between cases and controls are shown in Table 4. From the three *RAGE* gene polymorphisms, eight possible haplotypes were acquired and those with a frequency >5% were analyzed. In haplotype-based case–control analysis, the overall frequency of haplotypes was significantly different among CTRL, CAD&HT and CAD patients (CTRL vs CAD&HT, $P=0.005$; CTRL vs CAD, $P<0.001$). The G-Gly-T haplotype was more prevalent in CAD&HT and CAD patients ($P=0.003$, $P_{corr}=0.024$; $P=0.002$, $P_{corr}=0.016$), whereas the G-Ser-T haplotype was more prevalent in CAD patients ($P<0.001$, $P_{corr}<0.008$) when compared with the CTRL group. Meanwhile, similar findings were obtained in analyses limited to those without a baseline diabetic status in both case groups.

The association of different haplotypes with HT, CHD&HT and CHD patients is shown in Table 5. Results from the haplotype-based logistic regression analysis indicated that compared with the reference T-Gly-T haplotype, the G-Ser-T haplotype was associated with an increased risk of CAD (OR, 1.670; 95% CI, 1.017–2.740; $P=0.043$), whereas the G-Gly-T haplotype was associated with a reduced risk of CAD (OR, 0.588; 95% CI, 0.395–0.874; $P=0.009$). In addition, viewing CAD as a subtype of CAD&HT, the haplotype-based analysis was also performed in the CAD+CAD&HT group and similar significant findings were observed in CAD&HT patients (G-Ser-T: OR, 1.671; 95% CI, 1.062–2.630; $P=0.026$; G-Gly-T: OR, 0.682; 95% CI, 0.480–0.968; $P=0.032$). Further analyses excluding those with baseline diabetes showed a similar significant association with the G-Ser-T haplotype, but not with the G-Gly-T haplotype.

Table 4 Association study with haplotypes consisting of a pairwise combination of three SNPs among HT, CAD, CAD&HT and CTRL patients

Haplotype	CTRL n=170	HT n=200	CAD&HT n=155	CAD n=175	χ^2P	CTRL vs HT P-value	P_{corr}	CTRL vs CAD&HT P-value	P_{corr}	CTRL vs CAD P-value	P_{corr}	CAD&HT vs CAD P-value	P_{corr}
G-Gly-C	29 (8.5%)	24 (6.0%)	37 (11.9%)	36 (10.3%)		0.492		0.325		0.385		0.907	
G-Ser-T	43 (12.6%)	45 (11.3%)	65 (21.0%)	88 (25.1%)		0.831		0.034	0.272	<0.001	<0.008	0.063	0.504
T-Gly-T	62 (18.2%)	61 (15.3%)	56 (18.1%)	76 (21.7%)		0.928		0.581		0.233		0.082	0.656
Global	338	380	307	347	<0.001	0.434		0.005		<0.001		0.253	

Without baseline diabetes

	n=170	n=126	n=96	n=138									
G-Gly-T	204 (60.0%)	159 (63.1%)	98 (51.0%)	121 (43.8%)		0.018	0.144	<0.001	<0.008	<0.001	<0.008	0.120	
G-Gly-C	29 (8.5%)	12 (4.8%)	23 (12.0%)	28 (10.1%)		0.008	0.064	0.692		0.895		0.484	
G-Ser-T	43 (12.6%)	29 (11.5%)	42 (21.9%)	73 (26.4%)		0.099	0.792	0.012	0.096	0.005	0.040	0.004	0.032
T-Gly-T	62 (18.2%)	41 (16.3%)	27 (14.1%)	52 (18.8%)		0.039	0.312	<0.001	<0.008	0.349		0.005	0.040
Global	338 (99.3%)	241 (95.7%)	190 (99.0%)	274 (99.1%)	<0.001	0.324		0.011		<0.001		0.251	

Abbreviations: CAD, coronary artery disease; CAD&HT, coronary artery disease combined with hypertension; CTRL, control; HT, hypertension; P_{corr}, P-value after Bonferroni correction; SNP, single-nucleotide polymorphism.
P-value for χ^2 statistical comparison of haplotype frequencies among CTRL, HT, CAD and CAD&HT patients.

Table 5 Logistic regression analysis with haplotype-based parameterization: haplotype T-Gly-T as the reference

Cardiovascular diseases	OR	95% CI	P-value	*χ^2P	Cardiovascular diseases without diabetes	OR	95% CI	P-value	*χ^2P
HT				0.008, 0.928					4.221, 0.040
G-Gly-T	1.246	0.836–1.856	0.281			1.179	0.755–1.841	0.470	
G-Gly-C	0.841	0.441–1.605	0.600			0.626	0.287–1.365	0.239	
G-Ser-T	1.064	0.615–1.839	0.825			1.020	0.552–1.885	0.950	
T-Gly-T	Reference	—	—			Reference	—	—	
CAD&HT				0.305, 0.581					12.998, 0.000
G-Gly-T	0.809	0.532–1.229	0.320			1.103	0.661–1.841	0.707	
G-Gly-C	1.413	0.771–2.589	0.264			1.821	0.896–3.703	0.098	
G-Ser-T	1.674	0.987–2.838	0.056			2.243	1.206–4.172	0.011	
T-Gly-T	Reference	—	—			Reference	—	—	
CAD				1.415, 0.234					0.875, 0.350
G-Gly-T	0.588	0.395–0.874	0.009			0.707	0.459–1.089	0.116	
G-Gly-C	1.013	0.560–1.832	0.967			1.151	0.609–2.176	0.665	
G-Ser-T	1.670	1.017–2.740	0.043			2.024	1.195–3.429	0.009	
T-Gly-T	Reference	—	—			Reference	—	—	
HT+CAD&HT				16.343, 0.000					0.277, 0.599
G-Gly-T	1.036	0.730–1.472	0.842			1.149	0.778–1.697	0.486	
G-Gly-C	1.115	0.650–1.910	0.693			1.100	0.604–2.006	0.755	
G-Ser-T	1.356	0.849–2.165	0.203			1.505	0.902–2.511	0.117	
T-Gly-T	Reference	—	—			Reference	—	—	
CAD+CAD&HT				24.089, 0.000					2.040, 0.153
G-Gly-T	0.682	0.480–0.968	0.032			0.843	0.574–1.236	0.381	
G-Gly-C	1.182	0.699–2.000	0.532			1.380	0.785–2.427	0.263	
G-Ser-T	1.671	1.062–2.630	0.026			2.099	1.295–3.402	0.003	
T-Gly-T	Reference	—	—			Reference	—	—	

Abbreviations: CAD, coronary artery disease; CAD&HT, coronary artery disease combined with hypertension; CI, confidence interval; HT, hypertension; OR, odds ratio.
P-value for logistic regression analysis with T-Gly-T as the reference haplotype to examine the relationship between haplotypes and research groups by further controlling for atherosclerosis risk factors as mentioned above.
*χ^2 and P-values were assessed for global likelihood-ratio test comparing the model with haplotypes to that without.

The association of *RAGE* G82S genotypes with atherosclerotic risk factors is shown in Table 6. It is noteworthy that there was a significant association between G82S genotypes and serum CRP ($P<0.001$) as serum CRP levels were significantly higher in subjects with the SS genotype ($9.8\pm2.2\,\mathrm{mg\,l^{-1}}$) compared with patients with the GS ($8.3\pm2.7\,\mathrm{mg\,l^{-1}}$) or GG ($4.9\pm2.4\,\mathrm{mg\,l^{-1}}$) genotypes. In addition,

Table 6 Influence of *RAGE* G82S genotypes on atherosclerotic risk factors

	GG (n=450)	GS (n=231)	SS (n=19)	F-value	P-value	R$_0$-value	P$_0$-value	r$_1$-value	P$_1$-value
SBP (mm Hg)	136.4 ± 29.3	135.4 ± 30.5	131.8 ± 35.8	0.244	0.784	−0.025	0.524	−0.018	0.638
DBP (mm Hg)	79.2 ± 16.9	79.0 ± 17.5	79.1 ± 20.1	0.015	0.985	−0.026	0.494	−0.012	0.765
CRP (mg l^{-1})	4.9 ± 2.4**,***	8.3 ± 2.7*,***	9.8 ± 2.2*,**	167.390	<0.001	0.601	<0.001	0.563	<0.001
TG (mmol l^{-1})	1.8 ± 1.3	1.8 ± 1.4	1.8 ± 1.0	0.060	0.942	0.000	0.997	0.002	0.956
TC (mmol l^{-1})	4.8 ± 1.2	4.8 ± 1.0	4.4 ± 0.7	2.958	0.061	0.007	0.857	−0.021	0.597
HDL-C (mmol l^{-1})	1.2 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	0.915	0.401	−0.019	0.631	−0.035	0.368
LDL-C (mmol l^{-1})	2.7 ± 0.8	2.7 ± 0.8	2.4 ± 0.6	0.951	0.387	0.014	0.711	0.004	0.923
FG (mmol l^{-1})	5.8 ± 3.5	5.7 ± 1.8	5.7 ± 1.8	0.080	0. 923	0.040	0.311	−0.023	0.573
LVEF (%)	57.8 ± 12.2	58.3 ± 12.2	55.8 ± 17.0	0.304	0.738	0.008	0.857	0.006	0.887
E/A	1.1 ± 0.6	1.0 ± 0.6	1.3 ± 0.8	1.203	0.301	−0.067	0.150	−0.035	0.457
LVIDd (mm)	47.7 ± 9.3	46.7 ± 8.0	49.2 ± 12.3	1.277	0.280	−0.021	0.603	−0.051	0.222
LVIDs (mm)	29.9 ± 11.3	28.3 ± 9.4	31.4 ± 14.5	1.931	0.157	−0.041	0.316	−0.066	0.111

Abbreviations: ANOVA, analysis of variance; CRP, C-reactive protein; DBP, diastolic blood pressure; *E/A*, conventional Doppler diastolic parameters; FG, fasting glucose; GG, major homozygotes for the G allele of G82S polymorphism; GS, heterozygotes for the S allele; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diameter at end-diastole; LVIDs, left ventricular internal diameter at end-systole; RAGE, receptor for advanced glycation end product; SBP, systolic blood pressure; SS, minor homozygotes for the S allele of G82S polymorphism; TC, total cholesterol; TG, triglyceride.
Values are expressed as mean values ± s.d., unless otherwise stated. Continuous variables were assessed by one-way ANOVA. Spearman's correlation analysis was performed for non-parametric variables. *P*-values for Spearman's correlation analysis both before (P_0) and after (P_1) adjusting for the potential covariates of age, gender, smoking status, alcohol use, medical treatment assignment and diabetes.
*$P<0.05$ vs GG; **$P<0.05$ vs GS; ***$P<0.05$ vs SS.

serum CRP levels were higher in individuals with the GS genotype compared with patients with the GG genotype. Furthermore, correlation analysis revealed that serum CRP levels showed a highly significant positive relation with the G82S polymorphism ($r_0=0.601$, $P<0.001$) and a similar correlation was maintained even after adjusting for the potential covariates of age, gender, smoking status, alcohol use, medical treatment and diabetes ($r_0=0.563$, $P<0.001$). Comparison of other risk factors with the *RAGE* G82S genotypes revealed no significant differences.

DISCUSSION

Our present data pertain to a genetic-association study investigating the possible pathophysiological involvement of *RAGE* gene variants in the development of CAD and HT. Taken together, our findings suggested that a haplotype bearing the G82S variant was significantly associated with an increased risk of CAD in patients with or without HT, but not in HT patients, although there was no difference between CAD and CAD&HT patients of S allele carriers in G82S. Meanwhile, the G82S variant was associated with increased serum CRP levels. The other two *RAGE* gene polymorphisms (−429T/C, 1704G/T) were not significantly associated with a risk of CAD or HT in patients.

Vascular inflammatory–immune reactions contribute vitally to the initiation and progression of vascular injury, thereby having key roles in the increased incidence and severity of atherosclerosis. Evidence suggests that AGEs contribute to the pathogenesis of atherosclerotic events, including CAD and HT. For instance, an elevated concentration of serum Nε-(carboxymethyl) lysine has been observed in CAD patients when compared with control subjects, with the highest levels existing in CAD&HT patients, supporting the premise that AGEs may not merely be 'innocent bystanders', but active participants in accelerating the development of CAD.[12] Further studies indicate that the effects of AGEs are mainly mediated by their specific cellular receptor, RAGE. Activation of RAGE induces a cascade of pathophysiological responses, thus imparting a potential impact on the cardiovascular system. For example, our previous studies found that AGEs can significantly increase the number of apoptotic bodies/nuclei in CMs in a time- and dose-dependent manner,[13] and, by virtue of their engagement of RAGE, increase the cytosolic free calcium

concentration in cultured neonatal rat cardiac myocytes. Furthermore, these effects are blocked by the addition of an antibody to RAGE.[14] Subsequent evidence reveals that RAGE is a multiligand, cell surface receptor that is upregulated in a diverse array of cell types, including glomerular epithelial cells (podocytes), endothelial cells, vascular smooth muscle cells and inflammatory mononuclear phagocytes and lymphocytes. Besides AGEs, ligands for RAGE include proinflammatory S100/calgranulins and amphoterin, high-mobility group box 1 and amyloid-β peptide, leading to the premise that even in euglycemia, ligand–RAGE interaction can propagate inflammatory mechanisms linked to chronic inflammatory cell perturbation and vascular injury.[15] For example, RAGE expression is highly upregulated in human atherosclerotic plaques, particularly in macrophages located at the vulnerable regions of atherosclerotic lesions that contain inflammatory mediators.[3] These considerations, together with our findings, highlight RAGE as an important receptor linked to chronic cardiovascular perturbation and tissue destruction.

Clearly, the upregulation and pathogenic effects of RAGE in vascular disease highlight *RAGE* gene variants as likely candidates involved in the pathogenesis of atherosclerosis. Polymorphic differences within key domains of the *RAGE* gene may alter gene expression and influence proinflammatory mechanisms, thereby inducing varied effects of RAGE in inflammatory settings. Regarding the G82S mutation, its location within the V-type immunoglobulin domain in the RAGE extracellular region has been illustrated to exhibit increasing receptor-binding affinity for RAGE ligands.[16] Previously, it was shown that ligands for RAGE engage the V-domain of the receptor and activate signal-transduction pathways that induce a cascade of pathophysiological responses, thus leading to vascular and inflammatory cell perturbation. An increasingly recognized standpoint of recent studies is that the *RAGE* G82S polymorphism can upregulate cellular inflammatory responses, resulting in the enhancement of proinflammatory mechanisms in immune/inflammatory diseases and vascular injury[6] that could potentially accelerate the development of CAD. In human studies, Jang *et al.*[8] have reported that subjects with the SS homozygous mutation of the G82S polymorphism had increased risk factors for cardiovascular disease, including increased serum CRP levels. Corroborating these studies, our present data suggested a significant

association of the haplotype carrying the G82S variant with an increased risk of CAD, thus providing extraordinary evidence for a harmful role of the *RAGE* exon gene mutation in the Chinese Han population. Next, a highly significant positive relationship between the G82S variant and serum CRP levels was observed as well. Atherosclerosis, currently regarded as a dynamic and progressive disease, can emerge as a result of endothelial dysfunction and inflammation. CRP, viewed as a key proinflammatory cytokine, seems to have a prominent role in accelerating endothelial dysfunction and subsequent atherothrombosis. As shown in our study, subjects carrying the SS genotype had remarkably higher levels of serum CRP compared with patients carrying the G allele, thus supporting a role for the 82S allele in heightening inflammatory responses. Meanwhile, *in vitro* experiments revealed that CRP, as noted previously, significantly increased RAGE protein levels through an upregulation of RAGE mRNA expression in endothelial progenitor cells. Furthermore, CRP augmented ROS production, altered antioxidant defenses and induced endothelial progenitor cell apoptosis.[17] Similar findings were also observed in human endothelial cells[10] and THP-1 cells.[18] In this manner, CRP may impair endothelial repair and magnify vascular inflammation, thus contributing further to the progression of vascular injury and the severity of atherosclerotic disease. In addition, our study showing significantly higher levels of serum CRP in CAD patients compared with those in HT patients and controls further highlights the implication that the G82S variant seems to contribute to CAD by functioning as a facilitator in the propagation of vascular inflammation and eventual atherosclerotic progression. These concepts are consistent with other studies identifying an increase in 82S allele distribution in diabetic subjects with microvascular dermatoses,[19] psoriasis vulgaris[20] and in patients with rheumatoid arthritis.[6]

However, previous genetic–epidemiological studies showed negative results from studies involving Caucasian subjects with cardiovascular disease on the grounds of low patient enrollment in the study or low allele frequency (~5%) of the 82S variant,[21] although the 82S allele seems to have a protective role in Korean CAD patients.[22] Clearly, inter-racial and ethnic differences in polymorphisms of genes may exist, affecting susceptibility to diverse diseases.[23] Common, complex diseases show an unknown mode of inheritance, and differing ethnicity, along with other factors, could confound the pathogenesis of CAD. We speculate that our positive results may be partly owing to the finding that 82S allele frequencies of the *RAGE* gene are statistically higher in the Chinese Han population compared with other countries and ethnic groups. It is important to note that the importance of including a haplotype-based method for assessment of genetic association is also appreciated in our study. Nonetheless, further investigation using a gene-based approach is warranted to evaluate the potential influences of the *RAGE* gene G82S polymorphism and the *RAGE* gene variation, in general, on cardiovascular disease progression and outcome in terms of ethnicity.

In addition, polymorphisms located in the transcriptional regulatory region of *RAGE* (−429T/C and 1704G/T) increased the transcriptional activity of *RAGE* and altered transcription factor binding *in vitro*. Furthermore, an association of the haplotype containing the −429T/C variant with a reduced risk of myocardial infarction was observed in a clinical study.[24] Other reports, however, did not support an association between variants (−429T/C, 1704G/T) of the *RAGE* gene and diabetic vascular diseases.[25,26] In this study, no correlations were found between alleles and/or haplotypes of the *RAGE* polymorphisms (−429T/C and 1704G/T) examined and risk of CAD. This negative result may have been due to a low frequency of alleles or numerous confounding factors associated with the development of

CAD, which may negate any influence that polymorphisms have on the expression and function of RAGE. To date, the pathophysiological role of these *RAGE* gene variants in accelerating the severity of CAD remains elusive, and further investigations must be performed.

Several limitations of our study merit consideration. First, our cohort consists of a middle-aged to elderly Chinese Han population, which limits the generalizability of our findings. Second, although the effects of atherosclerosis risk factors on the association between *RAGE* G82S and CAD are adjusted by multivariate analysis, there may be factors not taken into account that may have, at least in part, influenced our results. Finally, owing to the low allele frequency of the SNPs tested in this study and the relatively low number of subjects analyzed in each category, future studies may require a greater number of participants to confirm this possible association.

In conclusion, our study suggesting a significant association between the haplotype carrying the G82S polymorphism and an increased incidence of CAD in the Chinese Han population highlights the 82S variant of *RAGE* as an important risk factor for CAD, whereas this is not the case for HT. Meanwhile, our data show a remarkable association of the G82S variant with elevated serum CRP levels, implying that the prevalence of *RAGE* 82S allelic variation may influence susceptibility to CAD by affecting vascular inflammation. Further studies are necessary to substantiate these findings and elucidate the importance of inter-ethnic differences affecting the outcomes of the complex polygenic nature of cardiovascular disease.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

1   Ramasamy, R., Vannucci, S. J., Yan, S. S. D., Herold, K., Yan, S. F. & Schmidt, A. M. Advanced glycation end products and RAGE: a common thread in aging, diabetes, neurodegeneration, and inflammation. *Glycobiology* **15**, 16–28 (2005).
2   Hudson, B. I., Hofmann, M. A., Bucciarelli, L., Wendt, T., Moser, B., Lu, Y. *et al.* Glycation and diabetes: the RAGE connection. *Curr. Sci.* **83**, 1515–1521 (2002).
3   Cipollone, F., Iezzi, A., Fazia, M., Zucchelli, M., Pini, B., Cuccurullo, C. *et al.* The receptor RAGE as a progression factor amplifying arachidonate-dependent inflammatory and proteolytic response in human atherosclerotic plaques: role of glycemic control. *Circulation* **108**, 1070–1077 (2003).
4   Hudson, B. I. & Schmidt, A. M. RAGE: a novel target for drug intervention in diabetic vascular disease. *Pharm. Res.* **21**, 1079–1086 (2004).
5   Laki, J., Kiszel, P., Vatay, A., Blaskó, B., Kovács, M., Körner, A. *et al.* The HLA 8.1 ancestral haplotype is strongly linked to the C allele of −429T>C promoter polymorphism of receptor of the advanced glycation endproduct (RAGE) gene. Haplotype-independent association of the −429C allele with high hemoglobin(A1C) levels in diabetic patients. *Mol. Immunol.* **44**, 648–655 (2007).
6   Hofmann, M. A., Drury, S., Hudson, B. I., Gleason, M. R., Qu, W., Lu, Y. *et al.* RAGE and arthritis: the G82S polymorphism amplifies the inflammatory response. *Genes Immun.* **3**, 123–135 (2002).
7   Zhang, H. M., Chen, L. L., Wang, L., Liao, Y. F., Wu, Z. H., Ye, F. *et al.* Association of 1704G/T and G82S polymorphisms in the receptor for advanced glycation end products gene with diabetic retinopathy in Chinese population. *J. Endocrinol. Invest.* **32**, 258–262 (2009).
8   Jang, Y., Kim, J. Y., Kang, S. M., Kim, J. S., Chae, J. S., Kim, O. Y. *et al.* Association of the Gly82Ser polymorphism in the receptor for advanced glycation end products (RAGE) gene with circulating levels of soluble RAGE and inflammatory markers in nondiabetic and nonobese Koreans. *Metabolism* **56**, 199–205 (2007).

9   Ridker, P. M., Buring, J. E., Cook, N. R. & Rifai, N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. *Circulation* **107,** 391–397 (2003).

10 Zhong, Y., Li, S. H., Liu, S. M., Szmitko, P. E., He, X. Q., Fedak, P. W. *et al.* C-reactive protein upregulates receptor for advanced glycation end products expression in human endothelial cells. *Hypertension* **48,** 504–511 (2006).

11 Wang, C. H., Li, S. H., Weisel, R. D., Fedak, P. W., Dumont, A. S., Szmitko, P. *et al.* C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. *Circulation* **107,** 1783–1790 (2003).

12 Xu, D. L., Liu, Y. L., Meng, S. R., Weng, C. H., Liu, Z. Q. & Liu, Y. L. Changes in serum concentration of the advanced glycosylation end-product N epsilon-(carboxymethyl) lysine in patients with coronary heart diseases. *Chin. J. Geriatr. Cardiovasc. Cerebrovasc. Dis.* **2,** 161–163 (2000).

13 Zeng, P., Xu, D. L., Li, Z., Lai, W. Y. & Ren, H. Effects of advanced glycation end-products on cell cycle distribution and apoptosis in neonatal rat cardiac myocytes. *J. First. Mil. Med. Univ.* **23,** 9–11 (2003).

14 Shao, Y. H., Xu, D. L., Zeng, P., Lai, W. Y., Ren, H. & Jiang, R. Y. Transient cytosolic free calcium changes in cultured neonatal rat cardiac myocytes in response to advanced glycation end-product treatment. *J. First. Mil. Med. Univ.* **25,** 274–276 (2005).

15 Kim, W., Hudson, B. I., Moser, B., Guo, J., Rong, L. L., Lu, Y. *et al.* Receptor for advanced glycation end products and its ligands: a journey from the complications of diabetes to its pathogenesis. *Ann. NY Acad. Sci.* **1043,** 553–561 (2005).

16 Osawa, M., Yamamoto, Y., Munesue, S., Murakami, N., Sakurai, S., Watanabe, T. *et al.* De-N-glycosylation or G82S mutation of RAGE sensitizes its interaction with advanced glycation endproducts. *Biochim. Biophys. Acta* **1770,** 1468–1474 (2007).

17 Chen, J. F., Huang, L., Song, M. B., Yu, S. Y., Gao, P. & Jing, J. C-reactive protein upregulates receptor for advanced glycation end products expression and alters antioxidant defenses in rat endothelial progenitor cells. *J. Cardiovasc. Pharmacol.* **53,** 359–367 (2009).

18 Mahajan, N., Bahl, A. & Dhawan, V. C-reactive protein (CRP) up-regulates expression of receptor for advanced glycation end products (RAGE) and its inflammatory ligand EN-RAGE in THP-1 cells: inhibitory effects of atorvastatin. *Int. J. Cardiol.* **11733,** 1–7 (2009).

19 Kankova, K., Záhejsky, J., Marova, I., Muzík, J., Kuhrová, V., Blazková, M. *et al.* Polymorphisms in the RAGE gene influence susceptibility to diabetes-associated microvascular dermatoses in NIDDM. *J. Diabetes Comp.* **15,** 185–192 (2001).

20 Vasku, V., Kankova, K., Vasku, A., Muzík, J., Izakovicová, H. L., Semrádová, V. *et al.* Gene polymorphisms (G82S, 1704G/T, 2184A/G and 2245G/A) of the receptor of advanced glycation end products (RAGE) in plaque psoriasis. *Arch. Dermatol. Res.* **294,** 127–130 (2002).

21 Hofmann, M. A., Yang, Q., Harja, E., Kedia, P., Gregersen, P. K., Cupples, L. A. *et al.* The RAGE Gly82Ser polymorphism is not associated with cardiovascular disease in the Framingham offspring study. *Atherosclerosis* **82,** 301–305 (2005).

22 Yoon, S. J., Park, S., Shim, C. Y., Park, C. M., Ko, Y. G., Choi, D. *et al.* Association of RAGE gene polymorphisms with coronary artery disease in the Korean population. *Coron. Artery Dis.* **18,** 1–8 (2007).

23 Kaplan, J. B. & Bennett, T. Use of race and ethnicity in biomedical publication. *JAMA* **289,** 2709–2716 (2003).

24 Zee, R. Y., Romero, J. R., Gould, J. L., Ricupero, D. A. & Ridker, P. M. Polymorphisms in the advanced glycosylation end product-specific receptor gene and risk of incident myocardial infarction or ischemic stroke. *Stroke* **37,** 1686–1690 (2006).

25 Yoshioka, K., Yoshida, T., Takakura, Y., Umekawa, T., Kogure, A., Toda, H. *et al.* Relation between polymorphisms G1704T and G82S of RAGE gene and diabetic retinopathy in Japanese type 2 diabetic patients. *Intern. Med.* **44,** 417–421 (2005).

26 Xu, J. X., Xu, B. L., Yang, M. G. & Liu, S. Q. −429T/C and −374T/A polymorphisms of RAGE gene promoter are not associated with diabetic retinopathy in Chinese patients with type 2 diabetes. *Diabet. Care* **26,** 2696–2697 (2003).