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Original Article

Renin-angiotensin system gene polymorphisms and coronary artery disease in Saudi patients with diabetes mellitus

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Abstract: Polymorphisms of some genes of the renin-angiotensin system (RAS), such as angiotensinogen (*AGT*; M235T), angiotensin II type 2 receptor (*AT2*; C3123A), and angiotensin-converting enzyme (*ACE*; insertion/deletion (I/D)) are involved in the development and progression of coronary artery disease (CAD) in diabetic individuals. In the present study, we aimed to determine whether three polymorphisms, *AGT*-M235T, *AT2*-C3123A, and *ACE* I/D are associated with CAD in Saudi patients with type two diabetes mellitus (T2DM). In 266 patients with CAD (169 patients with T2DM and 97 without T2DM), restriction fragment length polymorphism analysis was used to detect polymorphisms in the three RAS genes. Within the CAD+T2DM group, for the *ACE* gene (I/D), homozygous DD was found in 65.68%, 25.44% carried the heterozygous ID, and 8.88% carried the homozygous II. Within the CAD-T2DM group, DD was found in 55.67%, 26.8% carried the ID, and 17.53% carried the II. The odds ratio (OR) of the *ACE* ID+DD *vs*. II was 2.18, with a 95% confidence interval (CI) of 1.04-4.60, and $P = 0.04$. Thus, we found an association between the *ACE* DD polymorphism and CAD in Saudi patients with T2DM, but not between the *AT2* C3123A and *AGT* M235T polymorphisms and CAD.

Keywords: Coronary artery disease, gene polymorphism, renin-angiotensin system, diabetes mellitus, Saudi Arabia

Introduction

The development and causes of coronary artery disease (CAD) have been investigated intensively in the last few decades. However, rapid advances in molecular biology have made it possible to identify gene polymorphisms that might affect or alter the function important proteins in the vascular system [1]. Among these genes, there are several attractive targets involved in the renin-angiotensin system (RAS), including gene variants in angiotensinogen (*AGT*), angiotensin I-converting enzyme (*ACE*), and angiotensin II type 1 receptor (*AT1*) [2]. The involvement of RAS in the regulation of the cardiovascular system has been well documented. RAS plays a pivotal role in the stimulation of vascular smooth muscle cell proliferation, inflammatory reactions, intimal fibrosis, and plaque calcification [3].

Most components of RAS are proteins and are susceptible to variations in structure, configuration, or quantity because of genetic variations and differences. Recently, the identification of a number of RAS genetic variants opened new avenues of research in different populations. Research has shown that the polymorphisms in certain components of RAS are involved in the development and progression of CAD in diabetic individuals [4]. This was supported by the efficacy of ACE inhibitors and angiotensin-II receptor blockers (ARBs) in preventing the progression and development of coronary atherosclerosis and related heart complications [5, 6]. ACE, a major component of RAS, converts the inactive angiotensin I (Ang I) to vasoactive angiotensin II (Ang II). Ang II is a potent vasopressor that influences the contractility and growth of the vascular endothelium and vascular smooth muscle cells (VSMCs), thereby playing an essential role in the development of coronary atherosclerosis, platelet activation and aggregation, activation of monocytes, and the synthesis of plasminogen activator inhibitor-1 [7].

The curative effects of agents acting on RAS components, particularly ACE and Ang II, in cardiovascular diseases suggest that the disease process might be affected by gene disorders. This was supported by association studies involving polymorphisms in the genes of RAS pathway components and coronary disorders. In addition, it has been reported that a 287 base-pair insertion/deletion (I/D) polymorphism in the intron of the *ACE* gene affects the levels of serum ACE [8]; thus providing a reasonable basis for the increased incidence of CAD in diabetic individuals. However, studies performed to determine the relationship between *ACE* polymorphisms and CAD in diabetic individuals from the different populations has been inconsistent [4, 9-11]. A disordered vascular endothelium is regarded as an important parameter in the pathogenesis of diabetic micro- and macro-angiopathy. Angiotensin II can enhance atherosclerosis by activating specific genes responsible for coronary calcifications in the vascular smooth muscle cells of coronary arteries [12].

Given this background, the present study aimed to explore whether polymorphisms in *AGT* (M235T), Angiotensin receptor II (*AT2*; C3123A), and *ACE* (I/D) are associated with susceptibility to CAD in Saudi Arabian patients with type 2 diabetes mellitus.

Materials and methods

Subjects

This study involved 169 (110 males and 59 females, mean age 60.41 ± 8.83 years) CAD patients with T2DM defined according to the World Health Organization (WHO) criteria. Patients with glycosylated hemoglobin (HbA_1c) ≥ 6.5% were considered to have T2DM. Meanwhile, 97 (66 males and 31 females, mean age 61.96 ± 10.02 years) individuals with HbA_1c<6.5% were considered nondiabetic. Patients were recruited from The Department of Cardiology, King Khalid University Hospital, Riyadh, Saudi Arabia. All subjects provided written informed consent for drawing blood at the time of angiography or at the time of screening, for research deoxyribonucleic acid (DNA) extraction to be used in studies approved by the hospital's institutional review board. The study was conducted in accordance with the guidelines set by the ethics committee of the College of Medicine and Research Centre (CMRC) of King Saud University, Riyadh, Saudi Arabia. All the subjects enrolled in this study were Saudi residents with similar dietary patterns. The key demographic data of the subjects were recorded, including age, gender, and lipid profile. Cardiologists assessed the CAD patients by reviewing their angiograms.

Ethical approval

The Institutional Review Board of the Ethics Committee at KKUH (King Khalid University Hospital) conducted this study after review and approval, and all subjects gave written informed consent before participation.

Sample collection and blood analysis

Blood samples for glucose, glycosylated hemoglobin, kidney, and lipid measurements were drawn from the patients after an overnight fast. The plasma glucose concentration was measured using the glucose oxidase method with a Biotrol kit (Biotrol, Earth City, MO, USA) on a Bayer Opera analyzer (Bayer Diagnostics [Siemens), Munich, Germany). Serum total cholesterol (TC) was measured using a Biotrol kit, and high-density lipoprotein cholesterol (HDL-C) was measured using a commercial Randox kit (Randox Laboratories Ltd., London, UK). Low-density lipoprotein (LDL) cholesterol (LDL-C) levels were calculated using the Friedewald formula and triglyceride (TG) levels were measured using the lipase/glycerol kinase UV endpoint method on the Opera analyzer. Serum creatinine (Creat) and urea (UREA) were measured using a Biotrol kit. The HbA_1C percentages were measured using an HbA_1c ion exchange DIALAB commercial kit (DIALAB Produktion und Vertrieb von chemisch, Wiener Neudorf, Austria).

DNA extraction

Genomic DNA was extracted from peripheral blood (in tubes containing EDTA as an anticoagulant) using a QIAamp DNA Isolation Kit from QIAGEN (Germany), according to the manufacturer's instructions.

Genotyping and polymorphism analysis

Genotyping of *AGT* (M235T), *AT2* (C3123A), and *ACE* I/D polymorphisms were determined using polymerase chain reaction-restriction fragment

Table 1. Demographic characteristics of the CAD patients with and without type 2 diabetes mellitus

Characteristic	Subjects			*p*-value
	Total	CAD+T2DM	CAD-T2DM	
	266	169	97	
Gender				
Male, %	176 (66.17%)	110 (65.09%)	66 (68.04%)	0.502
Female, %	90 (33.83%)	59 (34.91%)	31 (31.96 %)	
FBS, mmol/L				
Mean ± SD	8.31 ± 3.27	9.60 ± 3.32	6.07 ± 1.44	<0.0001
Range	(3.5-19.2)	(4.20-19.2)	(3.50-9.40)	
TC, mmol/L				
Mean ± SD	4.19 ± 0.94	4.19 ± 0.99	4.18 ± 0.84	0.930
Range	(0.77-7.5)	(0.77-7.30)	(2.40-7.50)	
TG, mmol/L				
Mean ± SD	1.91 ± 1.18	1.87 ± 1.06	1.97 ± 1.37	0.536
Range	(0.57-8.7)	(0.57-8.70)	(0.65-8.32)	
HDL-C, mmol/L				
Mean ± SD	1.13 ± 0.73	1.13 ± 0.84	1.12 ± 0.45	0.585
Range	(0.52-10.7)	(0.52-10.70)	(0.53-3.73)	
LDL-C, mmol/L				
Mean ± SD	2.36 ± 0.77	2.39 ± 0.77	2.31 ± 0.77	0.416
Range	(0.59-5.89)	(0.59-4.74)	(0.80-5.89)	
HbA_1c, %				
Mean ± SD	7.24 ± 1.57	8.22 ± 1.03	5.51 ± 0.37	<0.0001
Range	(5.0-12.4)	(5.80-12.40)	(5.0-6.50)	
CREA, μmol/L				
Mean ± SD	87.41 ± 16.77	86.50 ± 16.73	88.99 ± 16.81	0.245
Range	(33.0-150.0)	(33.0-145.0)	(38.0-150.0)	
UREA, mmol/L				
Mean ± SD	6.79 ± 4.36	6.90 ± 5.28	6.60 ± 1.88	0.504
Range	(2.2-13.4)	(2.6-7.1)	(2.2-13.4)	

Data represent the mean ± SDs for all quantitative traits. Student's *t*-test and the X^2 test were used to compare the values of diabetic and nondiabetic CAD patients. FBS: fasting blood glucose, TC: total cholesterol, TG: triglyceride, HDL-c: high-density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol, HbA_1c: glycosylated hemoglobin, CREA: creatinine, UREA: urea.

length polymorphism (PCR-RFLP) from genomic DNA. The primer sets were selected based on previously published information [6, 8, 13]. *AGT* (M235T), forward primer: 5'-CAG GGT GCT GTC CAC ACT GGA CCC C-3' and reverse primer: 5'-CCG TTT GTG CAG GGC CTG GCT CTC T-3'; *AT2* (C3123A), forward primer: 5'-GGA TTC AGA TTT CTC TTT GAA-3' and reverse primer: 5'-GCA TAG GAG TAT GAT TTA ATC-3'; and *ACE* I/D, forward primer: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3' and reverse primer: 5'-GAT GTG GCC ATC ACA TTC GTC AGT T-3'. Genomic DNA template (3 μL, 150 ng) was added to the PCR reaction mixture containing 12.5 μL of 2×Promega master mix, 2 μL of each primer, and distilled water to a final volume of 25 μL. The PCR conditions were an initial denaturation at 94°C for 2 min; followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min; and a final extension at 72°C for 2 min in a My Cycler (Bio-Rad). Digestion of the C3123A and M235T PCR products was performed by the addition of 1 μL of the appropriate restriction enzyme (A*lul* and Pf*lFl*: New England Biolabs Inc., UK) to 10 μL of PCR products in 2 μL of 10×buffer (final reaction volume = 20 μL). The mixture was centrifuged for 2 min at 1398×*g* and kept in a water bath at 37°C overnight. The resulting fragments were resolved by electrophoresis (80 V, 60 min) on 3.0% agarose gels and visualized directly under UV light. For *AGT* (M235T), the homozygous individuals for the M allele (MM genotype) were identified by the presence of a single 165-bp PCR product. Those homozygous for the TT allele (TT genotype) were identified by the presence of both 140-bp and 25-bp PCR products. The heterozygous individuals (MT genotype) were identified by the presence of 165-bp, 140-bp, and 25-bp PCR products. For *AT2* (C3123A), the homozygous individuals for the C allele (CC genotype) were identified by the presence of a single 321-bp PCR product. Those homozygous for the A allele (AA genotype) were identified by the presence of both 214-bp and 107-bp PCR products. The heterozygous individuals (CA genotype) were identified by the presence of 321-bp, 214-bp, and 107-bp PCR products. For *ACE* I/D, the homozygous individuals for the D allele (DD genotype) were identified by the presence of a single 190-bp PCR product. Those homozygous for the I allele (II genotype) were identified by the presence of a single 490-bp

Table 2. Clinical risk factors in CAD patients with and without type 2 diabetes mellitus

Parameter	CAD+T2DM	CAD-T2DM	OR	95% CI	*p*-value
	169	97			
Dyslipidemia					
Positive	108 (63.90%)	43 (44.33%)	2.99	(1.26-3.51)	0.004
Negative	61 (36.1%)	51 (55.67%)			
Hypertension					
Hypertensive	121 (71.6%)	64 (65.98%)	1.30	(0.76-2.22)	0.338
Normotensive	48 (28.4%)	33 (34.02%)			
Smoking					
Smoker	63 (37.28%)	39 (40.21%)	0.88	(0.53-1.47)	0.637
Nonsmoker	106 (62.72%)	58 (59.79%)			

CAD+T2DM, coronary artery disease with type 2 diabetes mellitus; CAD-T2DM, coronary artery disease without type 2 diabetes mellitus; OR, odds ratio; CI, confidence interval.

Table 3. *AGT* (M235T), *AT2* (C3123A), and *ACE* (I/D) genotype distributions in CAD patients with and without type 2 diabetes mellitus

Genotype	Subjects		X^2	*P*
	CAD+T2DM, n = 169	CAD-T2DM, n = 97		
AGT (M235T)				
MM	47 (27.81%)	22 (22.68%)	0.84	0.36
MT	70 (41.42%)	40 (41.24%)	0.38	0.54
TT	52 (30.77%)	35 (36.08%)	1.16	0.28
AT2 (C3123A)				
AA	71 (42.02%)	43 (44.33%)	0.14	0.71
CA	32 (18.93%)	12 (12.37%)	1.53	0.21
CC	66 (39.05%)	42 (43.30%)	0.03	0.86
ACE (I/D)				
II	15 (8.88%)	17 (17.53%)	4.34	0.04
ID	43 (25.44%)	26 (26.80%)	2.13	0.14
DD	111 (65.68%)	54 (55.67%)	4.84	0.03

The X^2 test was used to compare the genotype frequencies between the CAD+T2DM and CAD-T2DM groups.

PCR product. The heterozygous individuals (ID genotype) were identified by the presence of both the 190-bp and 490-bp PCR products.

Statistical analysis

The statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA). The measured data were summarized using the mean ± standard deviation (SD) and compared using a two-sample t-test. The enumeration count data were summarized as the number (%) and compared using a chi-square test (X^2 test). Two analyses were used to evaluate the allelic and genotypic frequencies, which were calculated from the observed genotypic counts and to assess the Hardy-Weinberg equilibrium expectations. The same methodology was applied to the comparisons between the allelic and genotypic frequencies. Associations were determined as odds ratios (ORs) and 95% confidence intervals (CIs). The odds of carrying a specific allele are defined as the frequency of subjects in whom the allele occurs divided by the frequency of subjects in whom the allele does not occur. An odds ratio for the genotype distributions was analyzed using the X^2 test.

Results

Demographic characteristics of the CAD patients with and without Type 2 diabetes mellitus

Details regarding the clinical and biochemical characteristics of the study population [169 CAD patients with type 2 diabetes mellitus (CAD+T2DM) and 97 CAD patients without type 2 diabetes mellitus (CAD-T2DM)] are included in **Table 1**. CAD patients with type 2 diabetes mellitus had significantly higher plasma levels of fasting blood sugar and blood levels of glycosylated hemoglobin ($P<0.0001$ for each) compared with the CAD patients without type 2 diabetes mellitus.

Clinical risk factors in the CAD patients with and without type 2 diabetes mellitus

Dyslipidemia, hypertension, and smoking were selected as major risk factors. The frequencies of the major CAD risk factors in T2DM patients are shown in **Table 2**. Dyslipidemia was present more frequently in the CAD+T2DM group than in the CAD-T2DM group. Thus, dyslipidemia was identified as a risk factor for CAD (OR = 2.99, $P<0.004$).

Table 4. *AGT* (M235T), *AT2* (C3123A), and *ACE* (I/D) allele frequencies in CAD patients with and without type 2 diabetes mellitus

Gene	Allele	CAD+T2DM, n (%)	CAD-T2DM, n (%)	OR (95% CI)	*p*-value
AGT (M235T)	M	164 (48.52%)	84 (43.30%)		
	T	174 (51.48%)	110 (56.70%)	1.23 (0.87-1.76)	0.245
AT2 (C3123A)	A	174 (51.48%)	108 (52.94%)		
	C	164 (48.52%)	96 (47.06%)	0.94 (0.67-1.34)	0.741
ACE (I/D)	I	73 (21.60%)	26 (30.93%)		
	D	265 (78.40%)	134 (69.07%)	1.42 (0.87-2.33)	0.164

T2DM+CAD: Coronary artery disease with type 2 diabetes; T2DM-CAD: Coronary artery disease without type 2 diabetes. Differences in the allele frequencies between diabetic patients with or without coronary artery disease were compared using Pearson's X^2 test and without adjusting for other covariates. Odds ratios with 95% confidence interval (95% CI) are presented.

Table 5. CAD odds ratio (OR) associations with *AGT* M235T, *AT2* C3123A, and *ACE* I/D genotypes

Genotype	OR	95% CI	*P*
AGT (M235T)			
MM			
MT	0.819	(0.43-1.55)	0.54
TT	0.695	(0.36-1.35)	0.28
MT+TT	0.761	(0.43-1.36)	0.36
AT2 (C3123A)			
AA			
CA	1.615	(0.75-3.47)	0.22
CC	1.05	(061-1.81)	0.86
CA+CC	1.099	(0.66-1.82)	0.71
ACE (I/D)			
II			
ID	1.874	(0.80-4.38)	0.15
DD	1.344	(0.67-2.69)	0.40
ID+DD	2.18	(1.04-4.60)	0.04

CI = confidence interval.

AGT (M235T), AT2 receptor (C3123A,) and ACE (I/D) genotype distributions in CAD patients with and without type 2 diabetes mellitus

The entire study population was genotyped for the three RAS-related gene polymorphisms: *AGT* (M235T) (alleles designated as M and T), *AT2* (C3123A) (alleles designated as C and A), and *ACE* (I/D) (alleles designated I and D). **Table 3** shows the genotype frequencies for the three polymorphisms in the CAD+T2DM and CAD-T2DM groups. The distributions of the genotypes were according to the Hardy-Weinberg equilibrium, as expected. Within the CAD+TDM group (n = 169), the MM AGT genotype was identified in 47 patients (27.81%), whereas 70 (41.42%) and 52 patients (30.77%) carried the MT and TT genotypes, respectively. Within the CAD-T2DM group (n = 97), the MM genotype was identified in 22 subjects (22.68%), whereas 40 (41.24%) and 35 subjects (36.08%) carried the MT and TT genotypes, respectively. For *AGT*, CAD patients with T2DM were more likely to carry the MM genotype than the CAD patients without T2DM, who had had higher frequencies of the MT and TT genotypes. For the *AT2* gene, the AA genotype was identified in 71 patients (42.02%) within the CAD+T2DM group, whereas 32 (18.93%) and 66 patients (39.05%) carried the CA and CC genotypes, respectively. Within the CAD-T2DM group, the AA genotype was identified in 43 patients (44.33%), while 12 (12.37%) carried the CA genotype and 42 patients (43.30%) carried the CC genotype. For *AT2*, CAD patients with T2DM had higher frequencies of the AA and CA genotypes compared with the CAD patients without T2DM. For the ACE (I/D) genotypes, the II genotype was found in 15 patients (8.88%) within the CAD+T2DM group, whereas 43 patients (25.44%) carried the ID genotype and 111 patients (65.68%) carried DD genotype. Within the CAD-T2DM group (n = 97), the II genotype was identified in 17 patients (17.53%) whereas 26 patients (26.80%) carried the ID genotype and 54 patients (55.67%) carried DD genotype. Higher frequency of the DD genotype and lower frequencies of the ID and II genotypes were observed in the CAD patients with T2DM compared to the CAD patients without T2DM. For ACE I/D polymorphism, the II and DD genotype frequencies deviated significantly between two groups (X^2 = 4.36, 4.84 and P = 0.04, 0.03 respectively).

AGT (M235T), AT2 receptor (C3123A), and ACE (I/D) allele frequencies in CAD patients with and without type 2 diabetes mellitus

Table 4 shows no significant differences in the observed (M, T), (A, C), and (I, D) allele distributions between the CAD with type 2 diabetes mellitus and the CAD without type 2 diabetes mellitus (P = 0.245, 0.741 and 0.164, respectively).

CAD odds ratio associations with AGT M235T, AT2 C3123A, and ACE I/D genotypes

The ORs of the *ACT* M235T genotype MM *vs*. MT, MM *vs*. TT and MM *vs*. MT+TT (95% CI) were 0.819 (0.43-1.55), 0.695 (0.36-1.53) and 7.61 (0.43-1.36), respectively, indicating that there was no significant association with CAD+T2DM disease. Similarly, the ORs of the *AT2* (C3123A) genotype AA *vs*. CA, AA *vs*. CC, and AA *vs*. CA+CC (95% CI) were 1.615 (0.75-3.47), 1.05 (0.61-1.81) and 1.099 (0.66-1.82), respectively, which indicated no significant association with CAD+T2DM disease. The ORs of the *ACE* I/D genotype ID *vs*. II and DD *vs*. II genotypes (95% CI) were 1.874 (0.80-4.38) and 1.344 (0.67-2.69), respectively, which showed no significant association with CAD+T2DM disease. By contrast, the OR of the ACE I/D genotype ID+DD *vs*. II genotype (95% CI) was 2.18 (1.04-4.60), which showed a significant association with CAD+T2DM disease (P = 0.04) (**Table 5**).

Discussion

In patients with T2DM, cardiovascular diseases are major complications, causing premature mortality [14]. Studies suggest that polymorphisms in the components of RAS are important in the development and progression of CAD in T2DM individuals [15]. Association studies of polymorphisms in the genes encoding RAS pathway components, such as ACE and Ang II, suggest their involvement in cardiovascular disease processes [4]. The assessment of the distributions of alleles and genotypes in cases and controls is considered a suitable method to study the role of genes in the development of multifactorial diseases [3].

The severity of, and susceptibility to, CAD seems to be related to the interplay among environmental, metabolic, and genetic factors [16, 17]. Male sex and advanced age have been proposed as risk factors for the development of CAD in T2DM individuals [18]. The association of RAS gene polymorphisms with classical risk factors, including hypertension, obesity, diabetes, and hyperlipidemia, has been reported [19-23]. In the present study, dyslipidemia was confirmed as a risk factor for CAD in a Saudi Arabian population. Our results are consistent with previous studies that reported that lipid metabolism disorders play an essential role in atherosclerosis progress in diabetic patients [4]. Hypertriglyceridemia is associated with elevated plasminogen activator inhibitor levels and reduced fibrinolytic activity, causing endothelial dysfunction, a precursor to CAD in diabetic individuals via enhanced oxidative stress [24]. Lowering LDL-c levels raises HDL-c levels and lowers triglyceride concentrations, which are associated with a much-reduced risk of cardiovascular disease in patients with diabetes [25].

In the present study, samples from patients with CAD and T2DM, and those with CAD without T2DM were investigated to assess the relationship between three RAS gene polymorphisms, CAD and DM in a sample of Saudi Arabian patients.

Angiotensinogen (AGT) is produced by the liver and cleaved into angiotensin I by renin, which is secreted by the kidney when the blood pressure lowers. Angiotensin I is then converted to angiotensin II, a potent stimulator of myocardial fibrosis and hypertrophy, which activates the sympathetic nervous system, stimulates fibroblast proliferation, and stimulates vasoconstriction [26]. The AGT M235T polymorphism is an attractive target when studying the genetic features of CAD because the distribution of *AGT* genotypes shows ethnic differences. Asian and African populations have higher frequencies of the T235 homozygote compared with Caucasian populations [27]. However, results concerning the association between the *AGT* polymorphism and CAD have been inconsistent [3]. In a previous study investigating the association of RAS gene polymorphisms with CAD in diabetic patients, a positive association of *AGT* 235T was found, suggesting that RAS activation might play a role in CAD development in subjects with type 2 diabetes [28]. In a Slovenian population, the presence of the *AGT* T235 homozygote was associated with a two-fold increase in myocardial infarction risk [29].

In the present study, among the genotypes of polymorphism *AGT* M235T (MM, MT and TT) there was no significant difference between the M and T alleles and no significant association with CAD disease was observed. 72% of the patients with CAD patients had conventional risk factors, such as age, sex, hypertension, diabetes, and smoking [26]. That study also found that the *AGT* M235T polymorphism was not related to the presence of CAD, and no *AGT* genotype differences were found in hyperten-

sion, diabetes, and male subgroup analyses [27]. The *AGT* M235T gene polymorphism was also not associated with susceptibility to CAD in Chinese patients with type 2 diabetes [3].

Ang II-induced oxidative stress plays an important role in Ang II-mediated cellular responses [4]. Ang II promotes atherosclerosis by activating genes related to coronary calcifications in the vascular smooth muscle cells of coronary arteries [12]. In humans, two subtypes of receptors serve as binding sites for Ang II: angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R, gene symbol *AT2*). The *AT2* gene is located on the X-chromosome and comprises three exons and two introns, with the entire open reading frame of the gene being situated in the third exon [30].

The results of the present study showed that CAD+T2DM were not associated with the *AT2* C3123A genetic polymorphism. No association was observed between the higher frequency of the AA genotype of the *AT2* C3123A polymorphism, and CAD among coronary artery disease depressed patients [31]. This might be because of the low expression of these variants in most populations studied, and these polymorphisms might become associated with CAD in studies with larger sample sizes. In Japanese men, the presence of the A allele of the C3123A polymorphism was observed to be associated with an increase in blood pressure, whereas carriers of the (C) allele did not show this association [32]. However, association has been reported between the *AT2* 3123 C/A gene polymorphism and cardiovascular diseases. Indeed, AT2R is involved in obesity-linked metabolic parameters. Thus, this polymorphism might be a polymorphic marker related to body mass index in women [33]. Moreover, polymorphisms in the *AT2* gene were associated with attenuated effects on blood pressure, as well as lipid profiles after weight loss. A study reported an association between glycemic control and (A) allele carriers of *AT2* in Japanese women. The *AT2* C3123A polymorphism is located in the 3' untranslated region (3' UTR) of the gene; therefore, the association with glycemic control parameters might be explained by linkage disequilibrium in a functional variant of the same or a different gene [34].

ACE plays an important role in RAS. Most evidence appears to show that the D allele is a risk factor for the development and progression of macro- or micro-vascular diabetic complications [3]. Our results showed a significant association between DD+ID genotype polymorphisms and CAD in type 2 diabetes mellitus. Our results are consistent with previous studies. The relationship between the *ACE* gene I/D polymorphism and the presence and severity of CAD, myocardial infarction (MI), and putative risk factors for coronary atherosclerosis was investigated in T2DM individuals [3]. That study suggested that the DD genotype of ACE is an independent risk factor for the development of CAD in type 2 diabetes. The DD genotype (*vs*. the II genotype) increased the risk of CAD in diabetes independently by 2.1-fold, while the ID genotype did not alter the risk significantly [3]. Data from previous studies strongly supported the independent association of the D allele of the *ACE* gene with CAD in Caucasian T2DM subjects [35, 36]. In an Indian population, a few studies have investigated the association between the *ACE* D polymorphism/CAD [37, 38] and I. A significantly increased frequency of ID and DD genotypes and the D allele in T2DM patients with multivessel CAD compared with those with stenosis vascular disease (SVD) confirmed by OR analysis, which agreed with results obtained in a Japanese population [4, 39]. A positive relationship between the number of stenosed coronary arteries and the presence of the D allele was demonstrated in a Caucasian population [40]. By contrast, some studies found no difference in the frequency of the D allele among patient groups with a varying number of affected vessels [41-43]. Klehe *ACE* I/D polymorphism is not associated with the occurrence of CAD in T2DM patients [44]. Similar studies in Taiwanese, Caucasian, and Chinese populations reported that the *ACE* D allele was not associated with glucose metabolism, T2DM, CAD, or MI [9, 45, 46]. No significant difference in the distribution of the *ACE* I/D polymorphism with respect to CAD was noted in T2DM subjects [47]. The genetic variation in the *ACE* gene (I/D variant) coincident with the dangerous effect of persistent hyperglycemia on the activation of circulating and tissue-specific RAS, with consequent metabolic dysregulation, possibly contributes towards an accelerated progression of CAD in T2DM patients [3]. In conclusion, we found an association between the *ACE* DD polymorphism and CAD in type 2 diabetes; however, we found no

association between the *AT2* C3123A and *AGT* M235T polymorphisms and CAD. Based on these results, Saudi Arabian diabetic patients with the DD genotype seem to be more prone to CAD. RAS gene polymorphisms have an important role in the development of CAD in patients with diabetes. Further studies with a larger study population, and including other RAS gene polymorphisms, are necessary to confirm our findings for diabetic patients with CAD.

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Disclosure of conflict of interest

None.

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