Impact of renin–angiotensin–aldosterone system polymorphisms on myocardial perfusion: Correlations with myocardial single photon emission computed tomography-derived parameters

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Background. **Renin–angiotensin–aldosterone system (RAAS) has an important role in atherosclerosis. We investigated the effects of six RAAS gene polymorphisms on myocardial perfusion.**

Methods and Results. **We examined 810 patients with known or suspected coronary artery disease (CAD) using stress–rest myocardial single-photon emission computed tomography. Summed stress score (SSS), summed rest score (SRS), summed difference score (SDS), transient ischemic dilation (TID), and lung/heart ratio (LHR) were recorded. The following gene polymorphisms were investigated: angiotensin-converting enzyme (*ACE*) insertion/deletion (I/D), angiotensinogen (*AGT*) M235T and T174M, angiotensin II type 1 receptor (*AT1R*) A1166C, renin (*REN*) C5312T, and angiotensin II type 2 receptor (*AT2R*) C3123A. The heterozygotes or homozygotes on *ACE* D allele were 7.54 times more likely to have abnormal SSS, while the *AGT* (T174M) heterozygotes were 5.19 times more likely to have abnormal SSS. The homozygotes of *ACE* D had significantly higher values on TID and LHR, while the *AGT* (T174M) heterozygotes had higher values on TID. The *AT1R* heterozygotes had greater odds for having SSS ≥ 3. The patients carried *AT1R* homozygosity of C allele had significantly higher values on TID, while heterozygotes of *AT1R* had significantly higher values on LHR.**

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Conclusions. **Among the polymorphisms investigated, *ACE* D allele had the strongest association with abnormal myocardial perfusion. (J Nucl Cardiol 2019;26:1298–308.)**

Key Words: ACE • angiotensinogen • angiotensin II receptors • CAD • Renin

Abbreviations	
ACE	Angiotensin-converting enzyme
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
MPI	Myocardial perfusion imaging
LBBB	Left bundle branch block
PCI	Percutaneous coronary intervention
PCR	Polymerase chain reaction
RAAS	Renin-angiotensin-aldosterone system
SNP	Single-nucleotide polymorphism
SPECT	Single-photon emission computed tomography

See related editorial, pp. 1309–1312

INTRODUCTION

Coronary artery disease (CAD) represents one of the main causes of death worldwide.[1,2] The role of genetic factors in CAD is still under investigation, but important advances have been achieved based on the modern genomic technologies.[3,4] Particularly, significant associations have been demonstrated between cardiovascular disorders and several genes involved in the renin–angiotensin–aldosterone system (RAAS).[5,6] Among the investigated genetic changes are the insertion/deletion (I/D) alleles of the angiotensin-converting enzyme (*ACE*) gene, and single-nucleotide polymorphisms (SNPs) observed in the angiotensin II type 1 receptor (*AT1R*), angiotensinogen (*AGT*), and renin (*REN*) genes.[5–13] On the other hand, little evidence is available regarding the cardiovascular effects of C3123A (rs11091046) polymorphism of the angiotensin II type 2 receptor (*AT2R*) gene.

Previously, we evaluated the associations between several SNPs, including *ACE* (I/D) polymorphism, with myocardial perfusion, using stress–rest myocardial perfusion single-photon emission computed tomography (SPECT).[14–16] In the present study, the effects of six RAAS-associated SNPs [*ACE* (I/D), *AGT* gene M235T (rs699) and T174M (rs4762), *AT1R* gene A1166C (rs5186), *REN* gene C5312T (rs12750834), *AT2R* gene C3123A (rs11091046)] on myocardial perfusion were investigated, based on SPECT-derived parameters.

MATERIALS AND METHODS

The sample consisted of 810 consecutive patients (76.4% males), who were prospectively enrolled in the study. The mean age of the participants was 61.6 ± 10.2 years (range 32 to 88 years). In total, between December 2015 and February 2017, 1517 patients were referred to the Department of Nuclear Medicine, University Hospital of Larissa (Larissa, Greece) and the Department of Nuclear Medicine, Army Share Fund Hospital (Athens, Greece) for a gated SPECT myocardial perfusion imaging (MPI) combined with stress testing, for the evaluation of known or suspected CAD and patient risk stratification. 660 patients were excluded from the study because they met one or more of the exclusion criteria, while 47 patients refused to participate in the study.

In our study, we excluded patients younger than 30 years, pregnant women, and patients with non-ischemic cardiomyopathy. Also, patients with marked arrhythmias were excluded from the study due to the possibility of gating errors that may produce perfusion abnormalities. Moreover, we excluded patients with a previous cardiac invasive procedure [coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI)], and patients with a history or other evidence of myocardial infarction, as they comprise an inhomogeneous group whose myocardial perfusion study is affected not only by myocardial ischemia but also by necrosis related to both episode severity and applied therapy.

Pharmacologic testing, using either adenosine or regadenoson, in combination with low-level exercise, was performed in 199 patients with contraindication to or inability to achieve a satisfactory exercise level. 36 patients with left bundle branch block (LBBB) or an implanted pacemaker underwent pharmacologic testing without low-level exercise.

Medications, such as β-blockers, calcium channel antagonists and nitrates, that could potentially affect patients' performance on stress testing, MPI and the related variables, were temporarily withdrawn (for approximately five half-lives), as previously described.[17,18]

Prior to testing, all patients gave informed consent for their complete participation, according to the University Hospital of Larissa and Army Share Fund Hospital ethical committees' guidelines and the ethical standards laid down in the 1964 Declaration of Helsinki. Data on symptoms, medications, previous cardiac events, coronary risk factors and cardiac or non-cardiac diagnoses were collected through a brief structured interview with each participant.

Hypertension was defined as a systolic blood pressure of 140 mmHg or greater at rest and/or a diastolic blood pressure of 90 mmHg or greater at rest, or treatment with antihypertensive medicines. Diagnoses of diabetes mellitus and lipid disorders were derived from the patients' medical documentation (when possible), the interviews with the patients, and the use of corresponding medications. Obesity was considered as a condition with body mass index (BMI calculated as weight in kilograms divided by height in meters squared) value of 30.0 or greater.

Prior to the study, patients were also given written information regarding radioprotection.

Genetic Analysis

Genomic DNA from peripheral blood samples was extracted using the QIAmp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Polymerase chain reaction (PCR) amplification for all SNPs was performed in an Eppendorf thermal cycler. PCR conditions were standardized and were the same for *AGT*, *REN*, *AT1R*, and *AT2R* genes. Genotyping for M235T and T174M polymorphisms of *AGT* gene, C5312T polymorphism of the *REN* gene, A1166C polymorphism of the *AT1R* gene, and C3123A polymorphism of the *AT2R* gene was carried out as previously described.[19–22] In addition, genotyping for *ACE* I/D polymorphism was carried out by PCR allowing the discrimination of II, ID, and DD genotypes of *ACE* gene, as previously described.[23] However, in order to avoid any ID to DD mistyping, each DD genotype was further confirmed through a second PCR.[24]

Stress Testing

After discontinuing cardio-active medication, 6- to 12-h fasting and avoiding smoking or engaging in heavy physical activity for at least 3 h before the procedure, patients underwent symptom-limited treadmill test (Bruce Protocol), as described previously.[17] Data on symptoms and estimated workload in metabolic equivalents (METs; using standard tables) were recorded.

Pharmacologic testing using adenosine or regadenoson, either with or without low-level exercise, was performed according to the European Association of Nuclear Medicine procedural guidelines.[17,25]

Gated SPECT Myocardial Perfusion Imaging

MPI gated-SPECT studies were performed using technetium-99m (^{99m}Tc) tetrofosmin (Myoview, GE Healthcare, USA). Acquisition and processing protocols used in our departments are in accordance with the EANM/European Society of Cardiology (EANM/ESC) procedural guidelines, and have been described in detail elsewhere.[17,18] All acquisitions were carried out in the supine position, without attenuation-scatter correction, using two different dual-headed SPECT cameras. The lung/heart ratio (LHR) was measured in the early post-stress images, as described previously.[18] Further, polar and three-dimensional mapping were carried out, and the transient ischemic dilation (TID) index was calculated (GE Xeleris software, USA, or Sopha Medical Vision software, France).

Acquired gated-SPECT datasets (8 frames per cardiac cycle, ± 20% heart rate acceptance window) were automatically analyzed to evaluate left ventricular function and calculate related parameters. Tomographic reconstruction was accomplished using filtered back projection with the Butterworth Filter. Left ventricular (LV) myocardium was divided into 17 segments for SPECT interpretation (using GE Xeleris software or Sopha Medical Vision Software).[26] Two independent experienced observers blindly evaluated the reconstructed images, the polar maps, and the three-dimensional images of both stress and rest studies. Radiotracer uptake was scored in each of the 17 segments, according to a 5-point scoring system (0: normal uptake; 1: mildly reduced uptake; 2: moderately reduced uptake; 3: severely reduced uptake and 4: no uptake).[26] If counts were decreased in a region and this was judged to be the result of attenuation artifact, the score was 0.[27] The view of a third observer was requested in 21 studies in which discordance between the two observers was detected, and the disagreement was resolved by consensus.[28] Ischemia was considered in every segment of LV myocardium with an uptake > 0 at stress imaging and a reduction of the score by, at least, one unit at rest. The summed stress score (SSS) and summed rest score (SRS) were calculated by adding the scores of the segments in stress and rest studies, and a summed difference score (SDS) was obtained by subtracting the SRS from the SSS to evaluate defect reversibility.[26] Studies with SSS < 3 were classified as normal; studies with SSS 3-8 were considered as mildly abnormal, those with SSS 9 to 13 moderately abnormal, while an SSS > 13 was considered as severely abnormal.[14,16,29]

Table 1. Demographic and clinical characteristics of the study cohort

Total (N = 810)	N (%)
Gender (men)	619 (76.4)
Age (years), mean (SD)	61.6 (10.2)
Smoking	170 (21.1)
Obesity	316 (40.2)
Hypertension	589 (72.8)
Lipid disorder	381 (47.6)
Diabetes	228 (28.4)
Use of b-blockers	110 (13.7)
Use of calcium-channel antagonists	73 (9.1)
Use of nitrates	59 (7.4)
Maximal heart rate, mean (SD)	136.7 (30.4)
Maximal systolic blood pressure, mean (SD)	179.8 (27.3)
Diastolic blood pressure	95.1 (11.0)
Metabolic Equivalents, mean (SD)	10.4 (2.7)
Angina during exercise testing	66 (8.3)
LHR, mean (SD)	0.46 (0.04)
TID index, mean (SD)	0.98 (0.07)
Myocardial perfusion SSS, mean (SD)	5.95 (4.14)
Myocardial perfusion SRS, mean (SD)	1.41 (1.85)
Myocardial perfusion SDS, mean (SD)	4.58 (3.72)

LHR, lung/heart ration; *SD*, standard deviation; *SDS*, summed difference score; *SRS*, summed rest score; *SSS*, summed stress score; *TID*, transient ischemic dilation

Statistical Analysis

Continuous variables are presented as mean ± standard deviation (SD). Student's *t* tests and analysis of variance (ANOVA) were used to compare mean SSS and SDS values between the different categories of genotypes. In order to investigate the independent associations of genotypes with SPECT variables, TID and LHR, linear regression models were performed after adjusting for age, sex, smoking, the presence of chest pain, diabetes, hypertension, obesity, lipid disorder, the use of cardio-active medications (β-blockers, calcium channel antagonists, nitrates), exercise duration, maximal systolic blood pressure, maximal heart rate, METs, double product, angina and abnormal ST response during exercise testing. Regression coefficients with their standard errors (SE) along with standardized regression coefficients are presented from the results of the linear regression analyses. Multiple logistic regression analysis was also performed in order to evaluate the independent association of genotypes with SSS ≥ 3. Adjusted odds ratios with 95% confidence intervals were computed from the results of the logistic regression analyses. All reported p values are two-tailed. Statistical significance was set at $p < 0.05$ and analyses were conducted using SPSS statistical software (version 19.0).

RESULTS

Demographic and clinical characteristics of the study group are shown in Table 1. Hypertension (72.8%), lipid disorder (47.6%), and obesity (40.2%) were the more prevalent risk factors. 685 patients (84.6%) had an abnormal myocardial perfusion SPECT (SSS ≥ 3). Specifically, 497 patients (61.4%) had a mildly abnormal SPECT study (SSS: 3 to 8); 154 patients (19.0%) had a moderately abnormal study (SSS: 9 to 13), and 34 patients (4.2%) had a severely abnormal study (SSS > 13).

The *ACE* I/D genotype distribution was 20.1, 44.3, and 35.6% for I/I, I/D, and D/D, respectively. The genotype A/A of the *AT1R* (A1166C) polymorphism

Table 2. Distribution of the different genotypes in the entire study group and according to SSS values

	Total N = 810 N (%)	SSS < 3 N = 125 N (%)	SSS 3 to 8 N = 497 *N* (%)	SSS 9 to 13 N = 154 N (%)	SSS > 13 N = 34 N (%)
ACE (I/D)					
I/I	163 (20.1)	65 (52)	78 (15.7)	18 (11.7)	2 (5.9)
I/D	359 (44.3)	60 (48)	265 (53.3)	30 (19.5)	4 (11.8)
D/D	288 (35.6)	0 (0.0)	154 (31.0)	106 (68.8)	28 (82.4)
AT1R (A1166C)					
A/A	437 (54.0)	80 (64)	274 (55.1)	69 (44.8)	14 (41.2)
A/C	330 (40.7)	38 (30.4)	206 (41.4)	69 (44.8)	17 (50.0)
C/C	43 (5.3)	7 (5.6)	17 (3.4)	16 (10.4)	3 (8.8)
AT2R (C3123A)					
C/C	399 (49.4)	50 (40.0)	248 (50.2)	80 (51.9)	21 (61.8)
C/A	143 (17.7)	42 (33.6)	74 (15.0)	19 (12.3)	8 (23.5)
A/A	265 (32.8)	33 (26.4)	172 (34.8)	55 (35.7)	5 (14.7)
AGT (M235T)					
M/M	91 (11.2)	12 (9.6)	54 (10.9)	19 (12.3)	6 (17.6)
M/T	470 (58.0)	59 (47.2)	284 (57.1)	112 (72.7)	15 (44.1)
T/T	249 (30.7)	54 (43.2)	159 (32.0)	23 (14.9)	13 (38.2)
AGT (T174M)					
T/T	585 (72.2)	116 (92.8)	337 (67.8)	115 (74.7)	17 (50.0)
T/M	225 (27.8)	9 (7.2)	160 (32.2)	39 (25.3)	17 (50.0)
M/M	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
REN (C5312T)					
C/C	495 (61.1)	72 (57.6)	325 (65.4)	71 (46.1)	27 (79.4)
C/T	277 (34.2)	47 (37.6)	147 (29.6)	76 (49.4)	7 (20.6)
T/T	38 (4.7)	6 (4.8)	25 (5.0)	7 (4.5)	0 (0.0)

ACE, angiotensin-converting enzyme; *AGT*, angiotensinogen; *AT1R*, angiotensin II type 1 receptor; *AT2R*, angiotensin II type 2 receptor; *REN*, renin; *SDS*, summed difference score; *SRS*, summed rest score; *SSS*, summed stress score

was the most prevalent (54%), followed by A/C (40.7%) and C/C (5.3%). Concerning the *AT2R* (C3123A) polymorphism, the distribution of C/C, C/T, and T/T genotypes were 49.4, 17.7, and 32.8%, respectively. The majority of our patients carried the mutant allele (T) of *AGT* (M235T) genotype (58% in heterozygosity and 30.7% in homozygosity), while only 11.3% had the normal allele (M). The mutant allele (M) for *AGT* (T174M) polymorphism was present only in heterozygosity (27.8%), and the majority (72.2%) of the patients carried the normal allele (T). The normal allele (C) was the prevalent allele in *REN* (C5312T) polymorphic site (C/C: 61.1%, C/T: 34.2%), while a small number of the patients carried the mutant allele (T) in homozygosity (4.7%). The proportion of patients with the aforementioned genotypes according to SSS values is presented in Table 2. Figure 1 shows the proportion of patients with *ACE* genotype with regard to SSS values. Mean SSS and SDS values according to the presence of different genotypes are shown in Table 3. Mean SSS and SDS were significantly different according to *ACE* I/D, *AT1R* (A1166C), *AT2R* (C3123A), and *AGT* (M235T) categories (Figure. 2). Moreover, mean SSS was different between those carried the normal allele (T) and heterozygotes for *AGT* (T174M) polymorphic site.

In order to investigate the independent association of genotypes on SSS and SDS, multiple linear regression models were used (Table 4) after adjusting for age, sex, smoking, the presence of chest pain, diabetes, hypertension, obesity, lipid disorder, the use of cardio-active medications (β-blockers, calcium channel antagonists, nitrates), exercise duration, maximal systolic blood pressure, maximal heart rate, METs, double product, angina, and abnormal ST response during exercise testing. Both heterozygotes and homozygotes of *ACE* I/D and *AT1R* genotypes had significantly higher values on SSS and SDS. The homozygotes for *AT2R* (C3123A) polymorphic site had significantly lower values on SSS and SDS, while *AGT* (T174M) heterozygotes had higher values on SSS. Further, *AGT* (M235T) homozygotes had lower values on SSS and SDS. Any genotype of *REN* gene was not significantly associated with SSS and SDS. As defined from the standardized regression coefficients, *ACE* D and *AGT* (M235T) had the strongest association. A total score from the *ACE* I/D, *AT1R* (A1166C), *AT2R* (C3123A), *AGT* (M235T), and *AGT* (T174M) genotypes was generated by assigning 0 for normal, 1 for heterozygotes and 2 for homozygotes. This score reflecting the combination of all genotypes was tested in the regression analyses and a significant association was found with both SSS ($\beta = 0.75$, SE = 0.09, $p < 0.001$) and SDS ($\beta = 0.66$, SE = 0.08, $p < 0.001$).

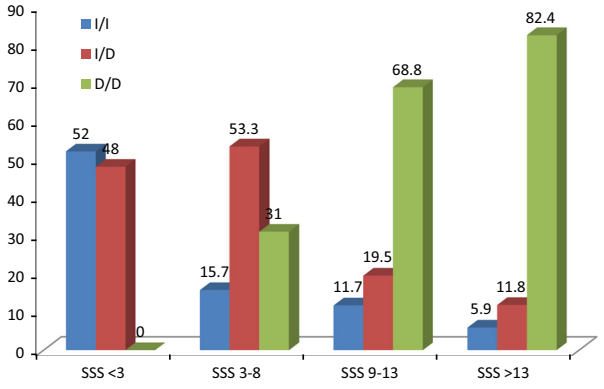

Figure 1. Proportions of patients being normal (I/I), heterozygotes (I/D), or homozygotes (D/D) for ACE polymorphic site according to SSS values.

Table 3. Mean SSS and SDS values according to the presence of different genotypes

	SSS			SDS		
	Mean	**SD**	***P***	**Mean**	**SD**	***P***
ACE (I/D)						
I/I	3.8	3.2	< 0.001[b]	2.7	2.7	< 0.001[b]
I/D	4.8	2.7		3.6	2.5	
D/D	8.6	4.7		6.9	4.3	
AT1R (A1166C)						
A/A	5.5	3.9	< 0.001[b]	4.1	3.5	0.011[b]
A/C	6.3	4.0		4.8	3.5	
C/C	8.2	6.1		5.9	5.8	
AT2R (C3123A)						
C/C	6.1	4.2	0.018[b]	4.7	3.5	0.021[b]
C/A	5.1	4.9		3.6	4.6	
A/A	5.9	3.7		4.8	3.5	
AGT (M235T)						
M/M	6.5	4.7	< 0.001[b]	4.7	2.9	< 0.001[b]
M/T	6.3	4		5	3.9	
T/T	5.1	4.1		3.8	3.5	
AGT (T174M)						
T/T	5.6	3.9	0.001[a]	4.4	3.4	0.074[a]
T/M	6.8	4.7		5	4.5	
REN (C5312T)						
C/C	5.9	4.2	0.842[b]	4.5	3.8	0.486[b]
C/T	6	4.2		4.7	3.7	
T/T	6.2	3.5		4	2.7	

ACE, angiotensin-converting enzyme; *AGT*, angiotensinogen; *AT1R*, angiotensin II type 1 receptor; *AT2R*, angiotensin II type 2 receptor; *REN*, renin; *SDS*,, summed difference score; *SRS* summed rest score; *SSS* summed stress score
[a]Student's *t* test
[b]ANOVA: analysis of variance

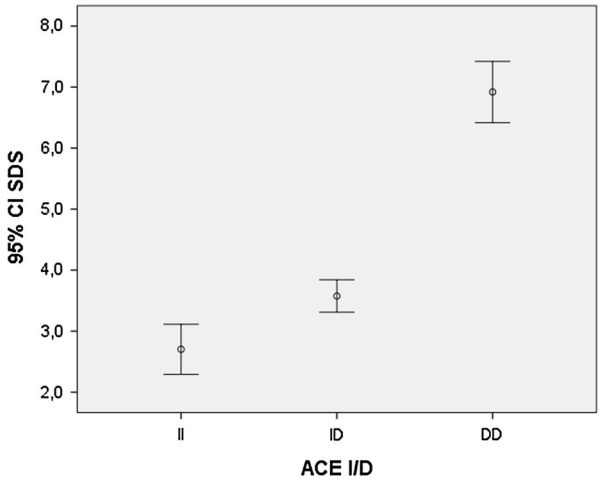

Figure 2. Mean SDS values according to ACE categories.

An analysis concerning rest and post-stress LV ejection fraction (EF) showed no significant associations with the study genotypes. The association of TID index and LHR with the aforementioned genotypes was also explored (Table 5) using multiple linear regression analysis. The homozygotes of D allele of *ACE* polymorphic site had significantly higher values on TID and LHR, while the homozygotes of A allele of *AT2R* polymorphic site had lower values on TID and LHR. Additionally, the patients carried *AT1R* homozygosity of C allele had significantly higher values on TID, while the heterozygotes of *AT1R* had significantly higher values on LHR. Both heterozygotes and homozygotes of *AGT* (M235T) genotype had significantly lower values on TID and LHR. The patients carried in heterozygosity the *AGT* (T174M) genotype had higher values on TID, and *REN* genotype was not significantly associated with TID or LHR.

Logistic regression analysis for SSS $\geq$ 3 was conducted after adjusting for all demographic and clinical data and the results are presented in Table 6. Subjects being heterozygotes or homozygotes for *ACE* (I/D) genotype were 7.54 times more likely to have abnormal SSS. The *AT1R* C/T heterozygotes had greater odds for having SSS $\geq$ 3, while *AT2R* heterozygotes had lower odds for having SSS $\geq$ 3. The association of *AGT* (M235T) genotype with abnormal SSS did not reach statistical significance, while the *AGT* (T174M) heterozygotes were 5.19 times more likely to have abnormal SSS.

Although we found a strong association between *ACE* D and abnormal SSS, patients carried this allele may also have other conventional cardiovascular risk factors. In particular, homozygotes of the *ACE* D allele were more likely to have hyperlipidaemia (52.5% vs. 45%, $p = 0.042$), obesity (46.7% vs. 36.7%, $p = 0.006$), hypertension (77.4% vs. 70.3%, $p = 0.031$), and diabetes (32.8% vs. 25.9%, $p = 0.041$). However, as mentioned above, the association between *ACE* D allele and abnormal SSS remained independent and strong, after adjusting for all other data, including cardiovascular risk factors.

20.2% of the study participants were asymptomatic (without chest pain) and from those 60% had abnormal SSS. Concerning *ACE* I/D polymorphism, 30.5% of the asymptomatic patients carried I allele in homozygocity, 39.6% were heterozygotes, and 29.9% carried D allele in homozygocity. Interestingly, the proportion of asymptomatic patients with abnormal SSS was significantly greater in I/D (63.1%) and D/D (73.5%) patients, as compared to I/I (42%) patients ($p = 0.005$).

Finally, the association of polymorphisms with end-systolic volume (ESV), end-diastolic volume (EDV), SRS, and left ventricular dyssynchrony was also investigated and no significant correlations were found ($p > 0.100$).

DISCUSSION

A combination of genotype variants of RAAS genes has been linked to myocardial ischemic events, and the subclinical progression of CAD.[30,31] MPI is a well-established, non-invasive imaging technique which has been extensively documented and supported in medical society guidelines, appropriateness criteria, and cost effectiveness position statements.[32,33] In the present study, we investigated the effects of six RAAS-associated SNPs on myocardial perfusion, based on SPECT MPI findings in patients with known or suspected CAD.

After adjusting for all demographic and clinical data, abnormal SSS was significantly associated with the D allele of *ACE* gene, in accordance with our previous report.[14] Further, the homozygotes of *ACE* D allele had significantly higher values on LHR and TID. Notably, asymptomatic patients carried D allele, were more likely to have abnormal SSS compared to homozygotes of I allele. Given the strong association between the ACE polymorphism and abnormal SSS, this finding may be important for the proper management of asymptomatic patients. The heterozygotes and homozygotes of *AT1R* genotype had significantly higher SSS and SDS values, while heterozygotes had greater odds for having abnormal SSS. Moreover, homozygotes of *AT1R* genotype had significantly higher TID values, while heterozygotes had significantly higher LHR values. The *AGT* (T174M) heterozygotes had higher values on SSS, whereas the association between *AGT* (M235T) polymorphism and abnormal SSS did not reach statistical significance.

Table 4. Multiple linear regression models: regression coefficients (β) and SE values for SPECT variables

	SSS				SDS			
	β[a]	SE	β[b]	*P*	β[a]	SE	β[b]	*P*
ACE (I/D)								
Normal, reference								
Heterozygotes	1.30	0.62	0.15	0.037	1.23	0.56	0.16	0.030
Homozygotes	4.60	0.64	0.57	< 0.001	4.02	0.57	0.52	< 0.001
AT1R (A1166C)								
Normal, reference								
Heterozygotes	0.72	0.36	0.08	0.048	0.64	0.32	0.08	0.049
Homozygotes	2.10	0.74	0.11	0.005	1.76	0.67	0.11	0.009
AT2R (C3123A)								
Normal, reference								
Heterozygotes	− 2.66	0.87	− 0.22	0.002	− 2.02	0.80	− 0.19	0.012
Homozygotes	− 0.19	0.59	− 0.02	0.747	0.01	0.54	0.00	0.989
AGT (M235T)								
Normal, reference								
Heterozygotes	− 1.30	0.68	− 0.15	0.059	0.13	0.59	0.02	0.830
Homozygotes	− 2.67	0.79	− 0.26	0.001	− 2.35	0.68	− 0.27	0.001
AGT (T174M)								
Normal, reference								
Heterozygotes	1.14	0.38	0.12	0.003	0.65	0.34	0.08	0.060
REN (C5312T)								
Normal, reference								
Heterozygotes	0.33	0.53	0.04	0.534	0.10	0.34	0.01	0.774
Homozygotes	1.04	1.49	0.04	0.485	0.72	0.71	0.04	0.322

ACE, angiotensin-converting enzyme; *AGT*, angiotensinogen; *AT1R*, angiotensin II type 1 receptor; *AT2R*, angiotensin II type 2 receptor; *I/D*, insertion/deletion; *REN*, renin; *SDS*, summed difference score; *SRS*, summed rest score; *SSS*, summed stress score
[a]Adjusted for age, sex, smoking, chest pain, diabetes, hypertension, obesity, lipid disorder, use of cardio-active medications (b-blockers, calcium channel antagonists, nitrates), maximal systolic blood pressure, maximal heart rate, metabolic equivalents (METs), angina during exercise testing
[b]Standardized regression coefficient

Finally, the heterozygotes for *AGT* (T174M) genotype had higher TID values.

There are some discrepancies between our results, which are based on SPECT imaging, and a number of previous studies evaluating the associations between RAAS SNPs and angiographic findings. Although the *ACE* (I/D), *AT1R* (A1166C), and *AGT* (T174M and M235T) polymorphisms have been associated with angiographically confirmed CAD, other researchers failed to confirm such associations.[34–47] These discrepancies may indicate the complexity of genetic effects on CAD development, rather than a failure in the demonstration of the underlying genetic associations. Nevertheless, even in the clinical setting, complete agreement between MPI and angiographic findings is neither expected, nor necessary, for patient management since coronary angiography provides evidence about the anatomical extent of the disease, while MPI evaluates the hemodynamic consequences of epicardial stenosis, endothelial function, small vessel function, and collateralization.[48]

Our study has some potential limitations. Attenuation-scatter correction was not applied since our departments are not equipped with SPECT/computed tomography (CT) systems. However, we tried to eliminate these artifacts through the application of gated SPECT techniques. Secondly, a direct correlation of the genotypes with angiographic results was not attempted since the investigation of such associations was above of the scope of our study. In any case, there was no clinical

Table 5. Multiple linear regression models: regression coefficients (β) and SE values for TID index and LHR

	TID				LHR			
	β[a]	SE	β[b]	*P*	β[a]	SE	β[b]	*P*
ACE (I/D)								
Normal, reference								
Heterozygotes	0.01	0.01	0.07	0.396	0.01	0.01	0.07	0.466
Homozygotes	0.04	0.01	0.23	0.005	0.02	0.01	0.26	0.006
AT1R (A1166C)								
Normal, reference								
Heterozygotes	0.00	0.01	− 0.03	0.656	0.02	0.01	0.18	0.010
Homozygotes	0.05	0.03	0.11	0.050	0.04	0.02	0.11	0.089
AT2R (C3123A)								
Normal, reference								
Heterozygotes	− 0.01	0.01	− 0.02	0.575	− 0.02	0.01	− 0.07	0.147
Homozygotes	− 0.02	0.01	− 0.12	0.002	− 0.01	0.004	− 0.09	0.031
AGT (M235T)								
Normal, reference								
Heterozygotes	− 0.05	0.01	− 0.31	< 0.001	− 0.02	0.01	− 0.23	0.014
Homozygotes	− 0.06	0.01	− 0.35	< 0.001	− 0.03	0.01	− 0.30	0.001
AGT (T174M)								
Normal, reference								
Heterozygotes	0.03	0.01	0.15	0.012	0.00	0.01	0.03	0.697
REN (C5312T)								
Normal, reference								
Heterozygotes	0.01	0.01	0.05	0.235	0.01	0.01	0.11	0.096
Homozygotes	0.01	0.03	0.01	0.823	0.01	0.02	0.06	0.432

ACE, angiotensin-converting enzyme; *AGT*, angiotensinogen; *AT1R*, angiotensin II type 1 receptor; *AT2R*, angiotensin II type 2 receptor; *I/D*, insertion/deletion; *LHR*, lung/heart ratio; *REN*, renin; *TID*, transient ischemic dilation
[a]Adjusted for age, sex, smoking, chest pain, diabetes, hypertension, obesity, lipid disorder, use of cardio-active medications (b-blockers, calcium channel antagonists, nitrates), maximal systolic blood pressure, maximal heart rate, metabolic equivalents (METs), angina during exercise testing
[b]Standardized regression coefficient

indication for the performance of coronary angiography in a significant number of participants, mainly those with normal or mildly abnormal MPI findings. Thirdly, the study sample consisted of patients who had been referred to our departments for a clinically indicated MPI. For this reason, the study sample had some special characteristics, such as high proportion of obese or hypertensive patients, and low proportion of definitively normal MPI studies. Although we enrolled the participants consecutively in order to avoid selection biases, we have to mention that the extrapolation of our findings to other populations needs further investigation. Finally, we performed MPI studies with ^{99m}Tc-tetrofosmin given the better image quality and lower radiation burden to the patients, despite the fact that thallium-201 can identify myocardial viability more accurately. In any case, we have to add that patients with history or other evidence of myocardial infarction were excluded from the study.

NEW KNOWLEDGE GAINED

To the best of our knowledge, this is the first study investigating the influence of six RAAS gene polymorphisms on myocardial perfusion based on MPI findings, in patients with known or suspected CAD. A better understanding of CAD predisposition and development may be achieved through the analysis of certain RAAS genotypes in combination with myocardial perfusion assessment. The identification of additional prognostic

Table 6. Odds ratios and 95% confidence intervals from multiple logistic regression analysis for the prediction of SSS $\geq$ 3 from the study genotypes

	OR (95% CI)[a]	*P*
ACE (I/D)		
Normal	1.00	
Heterozygotes/homozygotes[c]	7.54 (4.77–11.90)	< 0.001
AT1R (A1166C)		
Normal		
Heterozygotes	1.63 (1.05–2.53)	0.029
Homozygotes	0.98 (0.40–2.37)	0.956
AT2R (C3123A)		
Normal		
Heterozygotes	0.49 (0.28–0.83)	0.008
Homozygotes	1.02 (0.62–1.66)	0.949
AGT (M235T)		
Normal		
Heterozygotes	1.00 (0.51–1.94)	0.988
Homozygotes	0.55 (0.28–1.10)	0.091
AGT (T174M)		
Normal		
Heterozygotes	5.19 (1.25–21.48)	0.023
REN (C5312T)		
Normal		
Heterozygotes	0.83 (0.56–1.24)	0.375
Homozygotes	0.94 (0.38–2.34)	0.900

ACE, angiotensin-converting enzyme; *AGT*, angiotensinogen; *AT1R*, angiotensin II type 1 receptor; *AT2R*, angiotensin II type 2 receptor; *I/D*, insertion/deletion; *REN*, renin; *SSS*, summed stress score
[a]Adjusted for age, sex, smoking, chest pain, diabetes, hypertension, obesity, lipid disorder, use of cardio-active medications (b-blockers, calcium channel antagonists, nitrates), maximal systolic blood pressure, maximal heart rate, metabolic equivalents (METs), angina during exercise testing
[b]Standardized regression coefficient
[c]Indicates reference category

factors may lead to more efficient preventive interventions and screening methods. Further, clinical decision-making may be improved after proper modifications of treatment and follow-up strategies.

CONCLUSIONS

In the present study, we investigated the associations between six RAAS-related SNPs and myocardial perfusion, as assessed by gated SPECT MPI. Among these polymorphisms, *ACE* D allele had the strongest association with abnormal myocardial perfusion. However, further research is needed for the confirmation of the observed associations.

Disclosure

The authors declare that they have no conflict of interest.

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