Original Article

jraas

Journal of the Renin-Angiotensin-Aldosterone System
January-March 2017: 1–9

DOI: 10.1177/1470320316688774
journals.sagepub.com/home/jra
SAGE

Association of seven renin angiotensin system gene polymorphisms with restenosis in patients following coronary stenting

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Abstract

Background and objective: Percutaneous coronary intervention, despite being effective for coronary revascularization, causes in-stent restenosis due to neointimal hyperplasia in a large number of patients. The renin-angiotensin system is involved in neointimal hyperplasia. This study sought to evaluate seven gene polymorphisms of key renin-angiotensin system components, including angiotensinogen, angiotensin-converting enzyme and angiotensin II type 1a receptors, and their associations with in-stent restenosis in patients with coronary artery disease following coronary stenting.

Methods and results: Three hundred and fifty-two patients undergoing coronary drug-eluting stent implantation were recruited. Seventy-five patients (21.3%) were diagnosed as restenosis by angiography. Genotyping for angiotensin-converting enzyme insertion/deletion demonstrated a significant association of angiotensin-converting enzyme DD genotype with the occurrence of restenosis. Direct DNA sequencing revealed no association of angiotensinogen (M235T, G217A, G152A, G-6A, and A-20C) or angiotensin II type I receptor A1166C polymorphisms with in-stent restenosis. However, angiotensin II type 1a A1166C polymorphism was significantly associated with increased susceptibility to restenosis in a subgroup of patients aged more than 60 years.

Conclusion: Thus, our study suggests that genetic polymorphisms of angiotensin-converting enzyme insertion/deletion are associated with in-stent restenosis in coronary artery disease patients following coronary stenting.

Keywords

Coronary artery disease, restenosis, renin angiotensin system, single nucleotide polymorphism

Date received: 9 August 2016; accepted: 13 December 2016

Introduction

Percutaneous coronary intervention (PCI) is one of the major established treatment modalities of invasive revascularization for patients with coronary artery disease (CAD).[1] However, restenosis, a major clinical problem attributed to neointimal hyperplasia, limits the long-term efficacy of percutaneous coronary angioplasty. Although angiographic restenosis has been substantially reduced by stenting, especially the use of drug-eluting stents, it remains problematic in a subset of patients and certain lesions.[2] To date, the etiological basis of restenosis has not been completely understood.

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It is known that the occurrence of restenosis after PCI is mainly due to revascularization and neo-intimal growth histologically characterized by the excessive proliferation of vascular smooth muscle cells (VSMCs). The renin-angiotensin system (RAS) has been implicated in the development and progression of neointimal hyperplasia predominantly through its bioactive peptide, angiotensin II (Ang II).[3–5] Angiotensin-converting enzyme (ACE) converts angiotensin I (Ang I) to Ang II which promotes the migration and proliferation of VSMCs, causes vasoconstriction, and regulates expression of adhesion molecules via its major cellular receptor, the Ang II type 1 receptor (AT1R).

Genetic factors are also important for individual susceptibility in response to PCI. Genes regulating levels and activity of the RAS contribute to vasoconstriction and neointimal hyperplasia and are associated with restenosis following intracoronary stent placement.[6–9] Angiotensinogen (AGT) is the precursor for all angiotensin peptides. It has been reported that AGT M235T polymorphism was associated with the increased risk for systemic artery hypertension in Caucasian-Brazilians and that AGT M235T may be an independent risk factor for in-stent restenosis.[10] Additionally, other AGT promoter polymorphisms, including G217A and A-6G, were significantly associated with stroke in patients with atrial fibrillation.[11] However, there are very limited studies on the association of AGT gene variations with coronary restenosis.[12] Some studies have suggested ACE gene insertion/deletion (I/D) and AT1R A1166C polymorphisms as risk factors for coronary restenosis. Subjects carrying D allele of ACE I/D polymorphism may have increased risk of myocardial infarction (MI) in Asians and Caucasians.[13] Kaur et al. reported an association of the AT1R 1166A/C polymorphism with acute MI among north Indian patients, particularly if integrated with ACE I/D polymorphism and smoking.[14] However, other studies failed to confirm those findings.[9,15–19]

The present study evaluated the polymorphisms of genes encoding the key RAS components, including AGT, ACE and the AT1R receptor, and their association with restenosis in patients of the Chinese Han population after coronary artery drug-eluting stent (DES) implantation.

Methods

Patients

Among the six hundred and sixty-five hospitalized patients who underwent coronary angiography (CAG) for suspected CAD during our study period, 352 patients were prospectively recruited to this study, successfully underwent coronary DES implantation, and had a clinic follow-up for at least 18 months in our hospital.

Baseline patient characteristics, including age, sex, smoking history, alcohol consumption history, status of hypertension, diabetes, chronic obstructive pulmonary disease (COPD), valvular heart disease, atrial fibrillation, cardiac dysfunction, carotid atherosclerosis, and blood lipid levels were collected from medical records. Patients with secondary hypertension, history of aorta surgery, connective tissue disease, myocardiopathy, congenital heart disease, active cancer, infective endocarditis, syphilis, rheumatic heart disease (RHD), or chronic kidney disease (glomerular filtration rate (GFR)<60 ml/min) were excluded. Stent implantation and post-stenting therapy was performed according to routine clinical practice. A successful PCI procedure was defined as a residual stenosis less than 20% and thrombolysis in myocardial infarction (TIMI) flow grade III. Post-procedural medications included aspirin (100 mg/day) and clopidogrel (75 mg/day). After 12 months of treatment with clopidogrel and aspirin, all patients continued receiving aspirin. Treatment with an inhibitor to ACE or angiotensin receptor blocker (ARB), beta-blockers, statins, or other medications was at the discretion of attending physicians, who were blinded for study design and all analyses. The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Review Committee of Taizhou Hospital, Wenzhou Medical University, China. Written informed consent was obtained from all participants before inclusion in the study. The methods were carried out in accordance with the approved guidelines.

CAG and follow-up

CAG was performed by licensed clinicians. Two senior clinicians independently evaluated the results of the CAG to assess the degree of coronary artery stenosis. A stenosis was defined as a 50% or greater in the luminal diameter of coronary artery or its branches, and used for counting the number of lesions. The lesions involving left anterior descending branch, left circumflex artery or right coronary artery were considered as one-vessel disease (1 VD), while lesions involving left main coronary artery were considered as two-vessel disease (2 VD). Patients were stratified into three groups: 1 VD, 2 VD, and multiple vessels $\geqslant$3 VD, according to the number of vessels with obstructive lesions. The Gensini score was used to quantify the coronary stenosis. The coronary artery score is the sum of all segment scores (each segment score is segment weighting factor multiplied by a severity score). Severity scores assigned to the specific percentage luminal diameter reduction of the coronary artery segment are 32 for $\geqslant$99%, 16 for 90%−99%, 8 for 75%–90%, 4 for 50%–75%, 2 for 25%–50%, and 1 for <25%. The Gensini Score was calculated for all of the vessels for individual patients.

All patients were followed-up for at least 18 months. The follow-up included a medical visit at the outpatient clinic at 15 days and followed by once every one to two months on three occasions after stent placement. All patients were encouraged to improve their lifestyles, including smoking cessation and a healthy diet. Clinical

events were assessed on the basis of the information collected from hospital readmission records or an interview with the patients. For all patients presenting cardiac symptoms during the interview, at least one clinical and electrocardiographic evaluation was performed at the outpatient clinic. Restenosis was identified by symptoms or signs of ischemia (electrocardiographic evaluation or exercise testing) and confirmed by the presence of angiographic >50% diameter stenosis in the stented segment.

Blood sample collection and DNA extraction

Peripheral venous blood (2 ml) for each patient was collected in a tube containing Ethylenediaminetetraacetic acid (EDTA)-K2. Genomic DNA was extracted from leukocytes using the DNA extraction kit according to the manufacturer's instructions (Generay, Shanghai, China) and dissolved in Tris-EDTA buffer. The concentration and purity (A260/A280 ratio) of DNA were determined using a nucleic acid spectrometer (Bio-Rad, California, USA). Only DNA samples with an A260/A280 ratio of 1.7–2.0 were stored at −20°C for polymerase chain reaction (PCR) analysis.

PCR and direct sequencing for detection of AGT, AT1R and ACE I/D polymorphisms

Genomic DNA (0.1 μg) was amplified by PCR in a 25 μl reaction mixture, containing 2.5 μl of 10×PCR buffer, 0.5 μl dNTP (10 mM each), primers of upstream and downstream 0.25 μl (20 mmol/l each), Taq DNA polymerase 2 U and sterile double distilled water. For AGT and AT1R, DNA was amplified by preheating at 95°C for 2 min for initial denaturation followed by 35 cycles of denaturation at 94°C for 45 s, annealing at indicated temperature (Table 1) for 45 s, and an extension for 1 min at 72°C, and a final extension for 10 min at 72°C. The remaining DNA was purified and sequenced at Shanghai Sunny Biotechnology Company, Shanghai, China. For ACE I/D detection, PCR was performed by preheating at 95°C for 2 min for initial denaturation, followed by 30 cycles of denaturation at 94°C for 30 s, annealing for 45 s with the annealing temperature from 62°C decreased 1°C every cycle until reaching 54°C, and an extension for 1 min at 72°C, with a final extension for 10 min at 72°C. All PCR primers were designed using the Primer premier 5.0 software (PREMIER Biosoft International, California, USA) and commercially synthesized (Generay, Shanghai, China) (Table 1). The PCR specificity was confirmed by running PCR products on 1.8% agarose gel followed by ethidium bromide (EB) staining and gel imaging analysis.

Statistical analyses

The SPSS 18.0 statistical package was used for statistical analyses. Gene linkage disequilibrium was analyzed by

Table 1. Polymerase chain reaction primer sequence, annealing temperature and product sizes.

Gene	SNP	Reference SNP	Sense (5′-3′)	Anti-sense (5′-3′)	Annealing (°C)	Product size(bp)
AGT	G217A	rs5049	CCTGCAAACTTCGGTAAATG	AGCGGAAGAAGGAAGACCTGACCAT	54	531
	A-20C	rs5050				
	G-6A	rs5051				
	G152A	rs11568020				
	M235T	rs699	TCATGGTGGTGGGCGTGTT	CCAGGAGATGTGGGTTTC	54	618
AT1R	A1166C	rs5186	CCCCTCAGATAATGTAAGC	TGTGGCTTTGCTTTGTCT	51	262
ACE	I/D	rs4646994	CTGGAGACCACTCCCATCCTTTCT	GATGTGGCCATCACATTCGTCAGAT	62	490, 190

ACE: angiotensin-converting enzyme; AGT: angiotensinogen; AT1R: angiotensin II type I receptor; I/D insertion/deletion; SNP: single-nucleotide polymorphism.

SHEsis software (http://analysis.bio-x.cn/myAnalysis.php). Gene frequency was calculated for all genotypes or alleles, and the difference among groups was performed using multiple group comparison. Data for continuous variables were presented as mean value±standard deviation (SD). Depending on data types, non-parametric Mann-Whitney test or chi-square test were used for testing the differences among the groups. In addition to univariate analysis, multivariate Logistic regression analysis was performed to evaluate the correlation of coronary restenosis with geographic factors (gender and age), comorbidity (hypertension and diabetes), lifestyle (smoking and alcohol drinking), lipid profiling, renal function (creatinine) and RAS gene polymorphisms. For multivariate logistic regression analysis, odds ratio (OR) and its 95% confidence interval (CI) were calculated. In all analyses, $p<0.05$ was considered statistically significant.

Results

Baseline characteristics

Among the 352 CAD patients successfully undergoing coronary DES implantation, seventy-five patients (21.3%) had in-stent restenosis after Percutaneous transluminal coronary angioplasty (PTCA) and intracoronary stent, while 277 patients underwent CAG with no restenosis lesions or new lesions found. The baseline characteristics of the 352 CAD patients are shown in Table 2. Except for significant differences in the level of serum creatinine, glucose, and TC between the two groups ($p<0.05$), there were no differences in other parameters between patients with and without restenosis. Additionally, we did not note differences in the distribution of coronary artery branch as well as Gensini score between patients with and without restenosis.

Genotype and allele frequencies

Three combinations of ACE insertion/deletion polymorphisms, including II (490 bp/490 bp), ID (490 bp/190 bp) and DD (190bp/190bp), were confirmed by PCR amplification (Figure 1(a)). As shown in Table 3, in univariate analysis, the frequency of ACE DD genotype was higher in patients with, as compared to patients without, restenosis ($p=0.048$). The OR was 1.966 with a 95% CI of 0.998–3.872). The OR was 1.96 with a 95% CI of 0.99–3.87). The frequency of ACE I/D genotype was lower in patients with, as compared to patients without restenosis (OR 0.52, $p<0.05$). No difference in ACE I/I phenotype was noted between the two patient groups. In multiple logistic regression analysis, after adjusting for potential confounders, the ACE D/D polymorphism was an independent risk factor for restenosis (OR=4.3; 95% CI: 1.3–14.8; $p<0.05$).

Five genotypes for AGT (M235T, G217A, G152A, G-6A, and A-20C) were verified by direct DNA sequencing (Figure 1(b)). In single variate analysis, though the frequencies of M235T A/A (OR 2.91, $p=0.15$), G217A G/G (1.46, $p=0.19$) and G-6A G/G (OR 2.33, $0.05<p<0.1$) were higher in patients with, than those in patients without, restenosis, but neither reached statistical difference at $p\leqslant 0.05$ level (Table 3). Conversely, the frequencies of M235T G/G (OR 0.62, $p=0.218$) and G217A A/G (OR 0.68, $p>0.05$) phenotypes were lower in patients with, than those in patients without, restenosis (Table 3). Again, no statistical significance in either genotype was seen between the two groups.

Three genotypes for AT1R A1166C were seen in our patient population by direct DNA sequencing (Figure 1(b) and Table 3). In either group, A/A phenotype was predominant, whereas C/C phenotype was minor. In univariate analysis, there were no differences in individual genotypes between two groups. In contrast, after stratification for age, AT1R A1166C polymorphism was linked with restenosis. In patients aged 60 years or older, the frequency of A/C genotype was significantly higher in restenosis, that that in control, groups (18.5% vs 8%, $p<0.05$). Further, multiple logistic regression analysis further revealed the A/C genotype of AT1R A1166C as a risk factor for coronary restenosis in this subgroup (OR 5.78, 95% CI 1.30–25.65, $p<0.05$).

Additionally, Hardy–Weinberg equilibrium (HWE) of genotypes was tested by chi-square test. The distribution of the AGT, ACE and AT1R genotypes, except ACE I/D and AT1R A1166C ($p<0.05$), were compatible with those expected with the HWE ($p>0.05$). Further, the genotype frequencies of ACE I/D and AT1R A1166C followed Hardy–Weinberg equilibrium in the present CAD patients without restenosis ($p=0.156$ and $p=0.549$ respectively).

Multiple logistic regression analysis further revealed the A/C genotype of AT1R A1166C as a risk factor for coronary restenosis in this subgroup (OR=5.782; 95% CI: 1.303–25.657; $p<0.05$).

Linkage disequilibrium in ATG gene

Linkage disequilibrium is a nonrandom association of alleles at two or more loci and is also impacted by gene mutation. To evaluate the association in multiple ATG polymorphisms we performed linkage disequilibrium analysis for ATG gene polymorphisms using SHEsis software. As seen in Figure 2, M235T and G-6A alleles showed a strong linkage disequilibrium (D'=0.921, $r^2=0.719$). However, an incompact linkage disequilibrium was noted for G152A and G-6A (D'=0.894, $r^2=0.243$) (Figure 2).

Discussion

In this study, we found that the ACE deletion/deletion polymorphism was associated with the in-stent restenosis in CAD patients following PTCA and intracoronary

Table 2. General patient characteristics.

	Patients		*p* value
	With restenosis *n*=75	Without restenosis *n*=277	
Female, *n* (%)	16 (21.3)	72 (26.0)	0.408
Age (year)	65.3 ± 11.5	63.7 ± 11.6	0.480
Diabetes, *n* (%)	21 (28.0)	69 (24.9)	0.586
Unstable angina pectoris, *n* (%)	44 (58.7)	145 (52.3)	0.330
Acute myocardial infarction, *n* (%)	31 (41.3)	132 (47.7)	0.330
Involved branches			
1	18	78	0.473
2	28	86	0.064
⩾3	29	113	0.739
Multivessel disease, *n* (%)	57 (76.0)	199 (71.8)	0.473
Number of stents, *n*	1.68 ± 1.05	1.52 ± 0.88	0.133
Stented coronary vessel			
LAD, *n* (%)	58 (77.3)	158 (57.0)	0.001
LCx, *n* (%)	7 (9.3)	47 (17.0)	0.096
RCA, *n* (%)	19 (25.3)	93 (33.6)	0.065
Stented segment length (mm)	33.37 ± 20.75	31.69 ± 18.04	0.538
Stent diameter	3.00 ± 0.43	3.05 ± 0.42	0.571
Diameter stenosis (%)	92.71 ± 8.92	93.11 ± 7.63	0.670
Gensini score	32.97 ± 18.28	35.20 ± 16.37	0.276
Carotid atherosclerosis, *n* (%)	33 (75.0)[a]	174 (76.3)[b]	0.775
Lower limb arterial atherosclerosis, *n* (%)	27 (61.8)[a]	152 (67.3)[b]	0.449
Brain infarction, *n* (%)	9 (12.0)	22 (7.9)	0.271
Creatinine (μmol/l)	91.4 ± 25.8	83.2 ± 20.9	0.021
Blood glucose (mmol/l)	6.1 ± 1.7	6.7 ± 2.6	0.039
Triglyceride (mmol/l)	1.9 ± 1.1	2.0 ± 1.6	0.854
Total cholesterol (mmol/l)	4.2 ± 1.2	4.7 ± 1.2	0.008
High-density lipoprotein-cholesterol (mmol/l)	1.3 ± 0.6	1.3 ± 0.4	0.512
Low-density lipoprotein-cholesterol (mmol/l)	2.4 ± 0.9	2.6 ± 0.9	0.109
Current smokers, *n* (%)	42 (56.0)	157 (56.7)	0.916
Alcohol, *n* (%)	11 (14.7)	51 (18.4)	0.450
Diastolic blood pressure (mm Hg)	81.0 ± 12.64	81.2 ± 14.0	0.958
Systolic blood pressure (mm Hg)	134.0 ± 28.0	138.0 ± 21.7	0.375
Statin therapy, *n* (%)	75 (100)	277 (100)	1.000
ACEI therapy, *n* (%)	12 (16.0)	56 (20.2)	0.412
ARB therapy, *n* (%)	16 (21.3)	86 (31.0)	0.100
Beta-blocker therapy, *n* (%)	30 (40.0)	114 (41.2)	0.857
Calcium channel blocker, *n* (%)	8 (10.7)	21 (7.6)	0.408
Nitrates, *n* (%)	43 (57.3)	146 (52.7)	0.476

ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; LAD: left anterior descending; LCX: left circumflex artery; RCA: right coronary artery.
[a]*n*=44; [b]*n*=226.

drug-eluting stent implantation. AT1R A1166C polymorphism AC genotype was an additional independent risk factor for restenosis in patients older than 60 years.

ACE is the key enzyme converting Ang I to Ang II, which is involved in the migration and proliferation of VSMCs, vasoconstriction, deposition of extracellular matrix deposition, inflammation, and thrombosis through the binding to its major receptor, AT1R.[16,20] The ACE I/D polymorphisms are closely related to the circulating ACE levels and the activation status of intracellular ACE. The DD individuals have two-fold higher levels of plasma ACE as compared to the II individuals. Consistent with previous reports,[16,17] we found that the ACE D/D genotype was an independent risk factor for restenosis. Although other studies failed to confirm such association,[17,18] a recent meta-analysis demonstrated a significant association of ACE gene polymorphisms with coronary restenosis.[21] Thus, the conflicting findings among individual studies may result from variations in research approaches.

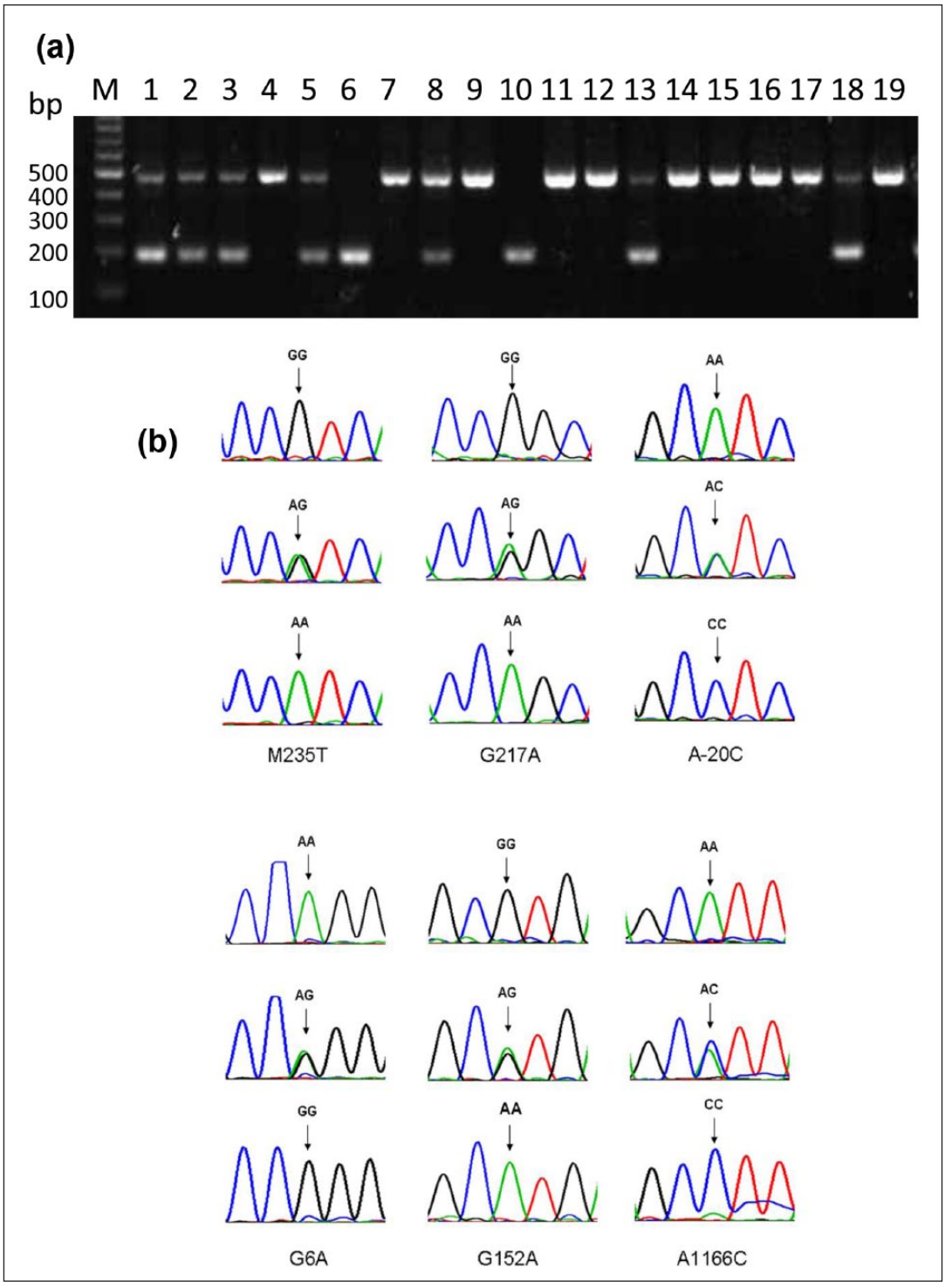

Figure 1. Representative images of polymorphisms for angiotensin-converting enzyme (ACE), angiotensinogen (ATG), and angiotensin II type 1 receptor (AT1R). (a) Electrophoresis images for ACE insertion/deletion (I/D) polymorphism. Lane M: 100 bp DNA marker; lanes 4, 7, 9, 11, 12, 14, 15, 16, 17, 19: genotype I/I; lanes 1, 2, 3, 5, 8, 13, 18: genotype of I/D; and lanes 6, 10: genotype D/D. (b) Individual polymorphisms of ATG (M235T, G217A, G152A, G-6A, A-20C) and AT1R (A1166C) genes identified by DNA sequencing. Each arrow indicates a single-nucleotide polymorphism.

We noted that the genotype frequencies of ACE I/D and AT1R A1166C were not in the Hardy-Weinberg equilibrium in CAD patients aged 60 years and with restenosis. This may result from the potential gene mutation of aging and chronic inflammation-mediated enhancement of reactive oxygen species or biological mediator generation in

Table 3. Genotype frequencies of the angiotensinogen (AGT), angiotensin-converting enzyme (ACE) and angiotensin II type I receptor (AT1R) polymorphisms.

Polymorphism	Genotype	Case (%)	Control (%)	OR (95% CI)	*p* Value
AGT M235T	G/G	23 (62.2)	123 (72.4)	0.628 (0.298–1.322)	0.218
	A/G	11 (29.7)	42 (24.7)	1.289 (0.587–2.831)	0.526
	A/A	3 (8.1)	5 (2.9)	2.912 (0.664–12.769)	0.155
AGT G217A	G/G	46 (68.7)	151 (59.9)	1.465 (0.825–2.602)	0.191
	A/G	18 (26.9)	88 (34.9)	0.685 (0.376–1.246)	0.213
	A/A	3 (4.4)	13 (5.2)	0.862 (0.238–3.116)	1.000
AGT G152A	G/G	61 (91.0)	236 (93.6)	0.689 (0.259–1.836)	0.454
	A/G	6 (9.0)	15 (6.0)	1.554 (0.579–4.173)	0.378
	A/A	0 (0.0)	1 (0.4)	/	/
AGT A-20C	A/A	54 (80.6)	195(77.4)	1.214 (0.619–2.381)	0.572
	A/C	13 (19.4)	55 (21.8)	0.862 (0.439–1.694)	0.667
	C/C	0 (0.0)	2 (0.8)	/	/
AGT G-6A	A/A	44 (65.7)	171 (67.9)	0.906 (0.513 –1.602)	0.734
	A/G	16 (23.9)	69 (27.4)	0.132 (0.065–0.268)	0.565
	G/G	7 (10.4)	12 (4.8)	2.333 (0.881–6.181)	0.080
ACE I/D	I/I	28 (38.9)	86 (31.7)	1.369 (0.799–2.345)	0.252
	I/D	29 (40.3)	153 (56.5)	0.520 (0.307–0.882)	0.014
	D/D	15 (20.8)	32 (11.8)	1.966 (0.998–3.872)	0.048
AT1R A1166C	A/A	56 (86.2)	222 (88.4)	1.230 (0.551–2.747)	0.613
	A/C	9 (13.8)	26 (10.4)	0.719 (0.319–1.620)	0.425
	C/C	0 (0.0)	3 (1.2)	/	/

CI: confidence interval; I/D: insertion/deletion; OR: odds ratio.

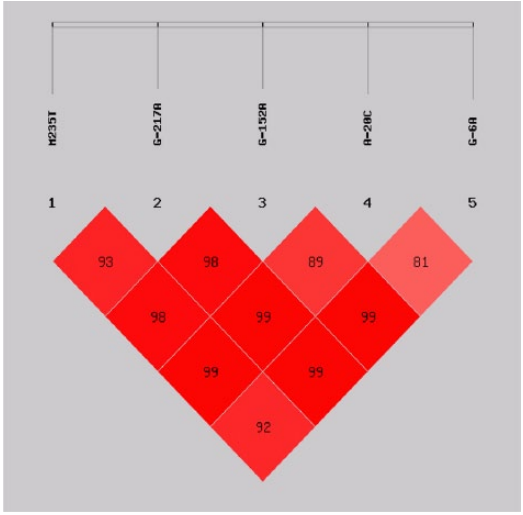

Figure 2. Pairwise linkage disequilibrium based on five angiotensinogen (AGT) single-nucleotide polymorphisms (SNPs) (M235T, G217A, G152A, G-6A, A-20C) using SHEsis software. Red squares represent high pairwise linkage disequilibrium, coloring down to white squares of low pairwise linkage disequilibrium. The numbers in the individual squares are D′ multiplied by 100. M235T and G-6A polymorphisms were in a strong linkage disequilibrium (D′=0.92, r^2=0.72).

this subset of patients. Alternatively, we cannot rule out the influence of the biases due to small sample size or subject selection in the present study.

AGT is the single precursor for Ang II, the main bioactive peptide in the RAS.[3] Most published studies on AGT polymorphisms and restenosis have focused on AGT M235T polymorphism, and findings are inconsistent. Some studies have suggested that M235T polymorphism is an independent risk factor for restenosis after stent angioplasty, whereas other studies have pointed out the role for the AT1R 1166A/C polymorphism in restenosis after PCI. Additionally, the influence of M235T polymorphism on restenosis after PTCA-balloon was dependent on whether patients have taken ACE inhibitors.[9,12,22,23] In this study, through a comprehensive analysis of five genotypes of AGT, none of the ATG genotypes (M235T, G217A, G152A, G-6A, and A-20C) was found to associate with coronary restenosis.

Ang II exerts its multi-cellular functions via AT1R, thus mutation(s) in AT1R can potentially influence the functional consequence of Ang II. Additionally, it has been shown that ARB treatment remarkably lowered the incidence of in-stent restenosis after stent implantation.[19] Thus, it is our assumption that genetic alternations in AT1R play an important role in post-PCI coronary restenosis. Thus, we compared the polymorphisms in the AT1R coding region between patients with and without restenosis. In agreement with previous reports,[9,15] our study found that the AC genotype of AT1R A1166C polymorphism was as an independent risk factor for restenosis in patients aged 60 years or older. Whether such an

association exists in patients aged less than 60 years needs to be further clarified.

Finally, the primary purpose of this study was to evaluate the association of RAS component gene polymorphisms with in-stent restenosis in patients with CAD following coronary stenting in a single institute. In our patients, whether these polymorphisms are associated with clinical manifestation and angiographic parameters were not determined. For example, further studies, particularly multiple center studies, are needed to examine the association of these gene polymorphisms with severity of atherosclerotic CAD.

In conclusion, we found that polymorphisms of ACE (D/D) and AT1R A1166C were associated with increased susceptibility to restenosis following coronary stenting. In contrast, no AGT genotype analyzed (M235T, G217A, G152A, G-6A, and A-20C) was significantly related to the incidence rate of restenosis in patients with coronary stenting. Further larger population studies will help confirm and generalize our findings.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the grants from the Zhejiang Provincial Natural Science Foundation of China (grant numbers LY14H020001, LY12H02002 and LY16H070008) and the National Natural Science Foundation of China (grant number 81400325).

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