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Original Article

Renin-Angiotensin-Aldosterone System (RAAS) Gene Polymorphism as a Risk Factor of Coronary In-Stent Restenosis

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Intimal proliferation is a main cause of in-stent restenosis. Over-excretion of angiotensin I converting enzyme (ACE) and aldosterone is reported to stimulate intimal hyperplasia and the genetic effect of these molecules may alter the process of in-stent restenosis. We hypothesized that the genetic polymorphisms that alter the expression of genes such as ACE I/D, CYP11B2-344C/T, and AGT M235T can affect in-stent restenosis. We analyzed the angiographic and clinical data of 238 patients (272 stents) who underwent coronary stenting and follow-up angiography, and analyzed the genotypes of ACE I/D, CYP11B2-344T/C, and AGT M235T. There was no significant difference in age, sex, or lipid profiles between the patent and restenosis groups. Diabetes mellitus was more frequent in the binary restenosis group. Quantitative computer-assisted angiographic (QCA) analysis revealed that the risk of in-stent restenosis increased with lesion length and was inversely proportional to post-stenting minimal luminal diameter (MLD) and reference diameter. There was no difference in the frequency of binary restenosis between genotypes in each of the three genes. However, follow-up MLD was significantly smaller in the ACE DD genotype than in the ACE II or ID genotypes. Defining restenosis as MLD < 2 mm, the restenosis rate was significantly higher in the ACE DD genotype than in the ACE II or ID genotypes. There was no significant synergistic effect between the three gene polymorphisms. In conclusion, while the ACE I/D polymorphism promoted the progress of in-stent restenosis and was of clinical significance, the other potential variables examined did not correlate with in-stent restenosis.

Key Words: In-stent restenosis, renin-angiotensin-aldosterone system, polymorphism

INTRODUCTION

Since the introduction of percutaneous balloon angioplasty and stent implantation for the treatment of coronary artery disease, restenosis has been recognized as the most important obstacle to favorable prognosis. Constrictive remodeling with adventitial scarring is the most important mechanism of restenosis following percutaneous balloon angioplasty. However, after stenting, neointimal hyperplasia is the main mechanism of restenosis.[1]

Genes related to the renin-angiotensin-aldosterone system (RAAS) were investigated as possible risk factors for cardiovascular and renal disease. Angiotensin I converting enzyme (ACE) is a core factor for the production of angiotensin II and the degradation of bradykinin. High

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activities of ACE increase the vessel wall thickness and cause thrombosis, constriction, and proliferation of smooth muscle cells.[2] The ACE I/D polymorphism appears to be a major determinant of circulating and tissue ACE, with ACE serum levels being higher in subjects homozygous for the D allele. Clinical studies involving genetic relationships have shown that the DD genotype is associated with an elevated risk of left ventricular hypertrophy (LVH) and myocardial infarction.[3,4] An association between D allele presence and angiographic in-stent restenosis has been reported in some previous studies.[5-8] Although the studies described above have shown a positive relation between polymorphism and restenosis, the relationship remains controversial.[9]

Aldosterone is a mineralocorticoid hormone, which via renal actions, controls sodium balance and intravascular volume, and thus helps to regulate blood pressure.[10] In addition, aldosterone may have several direct actions on the heart, including development of cardiac hypertrophy and fibrosis by stimulating cardiac collagen synthesis and fibroblast proliferation.[11,12] Variations in the aldosterone synthase (CYP11B2) gene may influence the activity of aldosterone.[13] This is particularly true of the cytosine/thymidine (C/T) exchange at position -344 in the regulatory region of the CYP11B2 gene, which is associated with the plasma and urinary levels of aldosterone.[14,15]

A molecular variant (M235T) of the angiotensinogen (AGT) gene exists in exon 2, consisting of a thymine-cytosine transition at nucleotide 704, which encodes threonine instead of methionine at residue 235 of mature AGT (T235/M235). Moreover, AGT M235T has been correlated to plasma AGT concentrations with circulating levels 15-40% higher in T235 homozygotes than in M235 homozygotes.[16,17] Recent studies have shown that the AGT M235T polymorphism is linked with hypertension, coronary atherosclerosis, and LVH via cellular proliferation.[16,18-20]

No study to date has been undertaken to investigate whether the AGT M235T and CYP11B2 -344T/C polymorphisms induce restenosis after stenting. Therefore, the aim of this study is to explore whether gene polymorphisms of RAAS influence the phenotypic expression of restenosis after stenting, either separately or synergistically, in a sample of Korean patients with coronary stenting.

MATERIALS AND METHODS

Materials

This study comprised 272 lesions in 238 consecutive patients with coronary artery disease who underwent coronary artery stent implantation between January 1998 and December 1999 at the Yonsei cardiovascular center, Yonsei University, Seoul, Korea. Follow-up angiography and blood sampling for genetic analysis were done in all subjects. According to follow up coronary angiography, the subjects were divided to a patent group (n=167) and a restenosis group (n=71). The patients that underwent stent implantation within 3 days of acute myocardial infarction were excluded to rule out the influence of thrombosis on restenosis. However, patients with unstable angina or stabilized old and recent myocardial infarction were included. Information about age, sex, body mass index (BMI), clinical diagnosis, serum lipid profile, and past history was obtained from medical records.

Assessment of angiographic data

Quantitative computer-assisted angiographic measurements (QCA) were performed on end-diastolic frames before balloon angioplasty, after stenting, and during follow-up coronary angiography using an on-line, quantitative, coronary angiographic system (Koroscop Top®/Hicor®, Siemens®, Munchen, Germany). Angiography was routinely performed in at least 2 projections, with follow-up angiography being performed using the same projections. Operators were unaware of the patients' genotype. Minimal luminal diameter (MLD), reference diameter, percent diameter stenosis, and lesion length were obtained from QCA. To define binary restenosis, two categorical approaches were adopted. One is the classic criteria of > 50% diameter stenosis during follow-up angiography.[21,22] The other is the criteria of <

2 mm of follow-up MLD. It has been reported that MLD ≥ 2 mm had a diagnostic accuracy of 89% in terms of identifying coronary flow reserve (CFR) ≥ 2.[23]

Genotyping

ACE gene polymorphism

Genomic DNA was extracted from 300 μL of whole blood with a QUIAmp Blood Kit (QUIAGEN®). The sequences of the sense and antisense primers were 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′, respectively. Polymerase chain reaction (PCR) was performed in a final volume of 20 μL, which contained 50 ng of genomic DNA, 10 pmol of each primer, 0.0625 mM dNTP, 1.0 mM $MgCl_2$, 50 mM KCl, 15 mM Tris-HCl (pH 8.3), and 0.4 U AmpliTaq® DNA. Amplification was performed with a 9600 Perkin Elmer Thermal Cycler. Samples were denatured for 10 minutes at 95℃ and then subjected to 35 cycles of 30 seconds at 92℃, 30 seconds at 67℃, and 1 minute at 72℃. The last extension was performed for 5 minutes at 72℃. PCR products were electrophoresed in 1.5% agarose gel and visualized directly by ethidium bromide staining. The insertion allele (I) was detected as a 490bp band and the deletion allele (D) as a 190bp band (Fig. 1). Insertion specific PCR (ISP) was performed to prevent underestimation of heterozygotes and overestimation of the D/D genotype. D/D and I/D types with an indistinct I band were subjected to a secondary, independent PCR amplification with a primer pair that recognized an insertion-specific sequence (5′-TGG GAC CAC AGC GCC CGC CAC TAC-3′; 5′-TCG CCA GCC CTC CCA TGC CCA TAA-3′), under identical PCR conditions.

CYP11B2 gene polymorphism

Sense and antisense sequences of the primer were 5′- CAG GAG GAG ACC CCA TGA GAC-3′ and 5′-CCT CCA CCC TGT TCA GCC C-3′, respectively. After an initial denaturation at 95℃ for 10 minutes, samples were cycled 35 times as follows: 1 minute at 95℃, 1 minute at 67℃, and 2 minutes at 72℃. The final extension was then performed for 5 minutes at 72℃. After amplification with annealing at 67℃, the PCR product was restricted with 5 U of the restriction endonuclease *Hae*III over 2 hours at 37℃, and the final product was electrophoresed in 2% agarose gel and visualized directly by ethidium bromide staining. The -344T allele lacks the *Hae*III site that is present in the -344C allele and gives rise to a fragment of 273bp rather than 202bp.

AGT M235T polymorphism

Sense and antisense sequences of the primer were 5′-CAC GCT CTC TGG ACT TCA CA-3′ and 5′-CAG GGT GCT GTC CAC ACT GGA CCC C-3′, respectively. After initial denaturation at 94℃ for 10 minutes, samples were cycled 35 times through the following steps: 15 seconds at 94℃, 45 seconds at 60℃, and 45 seconds at 72℃. The PCR product was incubated with *Tth*111/*Asp*I (Takara®) at 67℃ for 16 hours, and the final product was electrophoresed in 3% agarose gel and visualized directly by ethidium bromide staining. The PCR product of the M235T homozygotes was cleaved by *Tth*111/*Asp*I to yield a 140bp fragment. In the absence of the M235T variant, the 164bp amplification product remained intact. Both 164bp and 140bp fragments were apparent for heterozygotes.

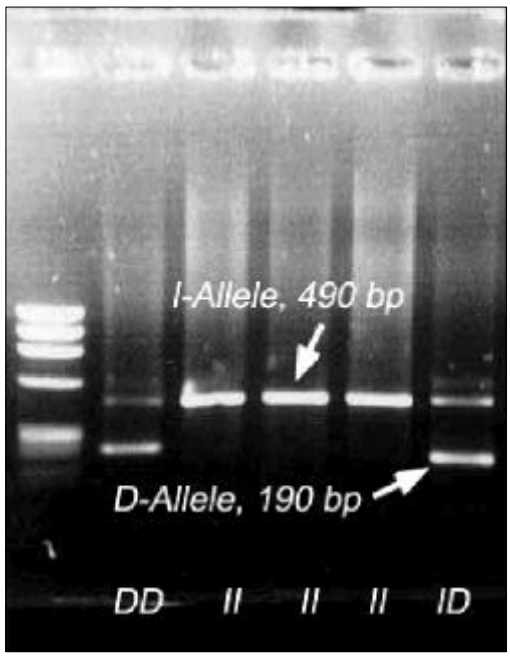

Fig. 1. Genotyping of ACE gene I/D polymorphism.

Statistical analysis

Clinical data, angiographic data and genotypes were compared for the patent and restenosis groups. Discrete variables were expressed as counts or percentages, and compared with Pearson's χ^2-test. Continuous variables were expressed as mean ± SD, and compared using independent 2-sided t-test or ANOVA for more than 2 groups. Independent association between the genotypes of each gene and outcome was assessed after adjusting for other potential confounding factors by multiple binary logistic regression analysis.

RESULTS

Characteristics of the subjects

The mean age of the patients was 59.5 ± 9.9 years, and 74.8% were men. The mean age did not differ significantly between the patent and restenosis groups. The male prevalence, smoking history, and hypertension were similar in both groups. However, the prevalence of diabetes mellitus (DM) was significantly higher in the restenosis group ($p < 0.05$). There were no significant differences of serum level of lipid (total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride) or BMI (Table 1).

Angiographic data

Of 272 stents, the patent and restenosis groups represented 73.2% and 26.8%, respectively. The prognosis of stenting was evaluated according to the characteristics of the lesions. In-stent restenosis was more frequent in lesions over 20 mm ($p < 0.01$). Restenosis was more frequent in angled lesion (> 45°), although not to a statistically

Table 1. Clinical Characteristics of the Patients

	Patent group (n=167)	Restenosis group (n=71)	*p* value
Age (years)	59.1 ± 10.6	59.8 ± 9.4	0.632
Male (%)	130 (77.8%)	48 (67.6%)	0.133
Past History			
DM	37 (22.2%)	26 (36.6%)	0.021
Hypertension	90 (53.9%)	36 (51.4%)	0.729
Smoking (PYS)	31.8 ± 19.5	33.6 ± 16.9	0.612
Total Chol (mg/dl)	199.1 ± 39.5	200.8 ± 39.3	0.417
HDL Chol (mg/dl)	44.6 ± 21.1	45.0 ± 30.3	0.91
LDL Chol (mg/dl)	121.1 ± 32.5	134.8 ± 85.8	0.088
TG (mg/dl)	156.6 ± 100.1	151.5 ± 81.3	0.715
Lp(a) (mg/dl)	37.5 ± 82.0	36.2 ± 28.2	0.943
Fibrinogen (mg/dl)	386.1 ± 143.7	387.0 ± 120.7	0.975
Angiographic Dx			
1 VD	95 (49.2%)	42 (53.2%)	
2 VD	58 (30.1%)	21 (26.6%)	
3 VD	37 (19.2%)	12 (15.2%)	
Left Main	3 (1.6%)	4 (5.1%)	0.311
Clinical Dx			
Stable angina	56 (29.0%)	19 (24.1%)	
Unstable angina	98 (50.8%)	44 (55.7%)	
MI (Recent & Old)	39 (20.2%)	16 (20.3%)	0.384

PYS, pack years; Chol, cholesterol; TG, triglyceride; VD, vessel disease; Dx, diagnosis; Lp(a), lipoprotein(a); MI, myocardial infarction.

significant degree, and neither did the presence of calcification and thrombus have a significant influence on the prognosis of stenting.

QCA revealed that the proximal reference, distal reference diameters, and post-stenting MLD were significantly greater in the patent group (p=0.011, 0.002, and 0.002, respectively). No significant difference was observed in pre-stenting MLD, percent diameter stenosis, or post-stenting residual stenosis between the two groups. Lesion length was significantly greater in the restenosis group (Table 2).

Genetic effects

Distribution of genotypes

The distributions of ACE II, ID, and DD genotypes were 33.6% (n=81), 43.6% (n=105), and 17.0% (n=41), respectively. These data are close to values reported in another study on Koreans.[24] The distributions of CYP11B2 -344 TT, CT, and CC genotypes were 42.3% (n=109), 42.3% (n=109), and 8.7% (n=21), respectively; data that are close to values reported in Japanese study.[25] In the case of AGT M235T polymorphism, the frequencies of each genotype were MM, 15.8% (n=38); MT, 29.9% (n=72); and TT, 49.4% (n=119); which are close to values reported in other studies with Japanese and Chinese.[26-28] The distributions of genotypes of all three gene polymorphisms are compatible with Hardy-Weinberg equation.

The effects of genetic polymorphism on restenosis

In the patent group, the distributions of II, ID, and DD genotypes were 33.5%, 49.2%, and 17.3%, and in the restenosis group they were 43.8%, 37.5%, and 18.8%, respectively (Table 3). There were no significant differences in genotype distributions between the two groups. Using binary multiple logistic regression analysis, adjustment was made for age, sex, DM, arterial hypertension, smoking, lesion length, and post procedural MLD. The adjusted odds ratio was 1.093 (95% confidence interval (CI), 0.344-3.470, p=0.881) for ID/

Table 2. Comparison of Morphological Characteristics and QCA Data of Stent Insertion Site between the Patent and Restenosis Groups

	Patent group (n=167)	Restenosis group (n=71)	p value
Lesion length			
Discrete (<10 mm)	64 (33.7%)	17 (24.3%)	
Tubular (10 - 20 mm)	78 (41.9%)	21 (30.0%)	0.007
Diffuse (>20 mm)	48 (25.3%)	34 (45.70%)	
Lesion angulations			
< 45°	180 (93.3%)	59 (85.5%)	
≥ 45° and < 90°	13 (6.7%)	87 (11.6%)	0.06
≥ 90°	0 (0%)	2 (2.9%)	
QCA Data			
Proximal RD (mm)	3.39 ± 0.57	3.14 ± 0.63	0.014
Distal RD (mm)	3.13 ± 0.63	2.76 ± 0.79	0.002
Pre MLD (mm)	0.83 ± 0.55	0.77 ± 0.43	0.43
Pre DS (%)	74.9 ± 15.3	74.2 ± 13.3	0.79
Post MLD (mm)	3.21 ± 0.53	2.9 ± 0.61	0.002
Post DS (%)	2 ± 8.8	1.7 ± 15.8	0.905
Lesion length (mm)	16.9 ± 6.97	20.55 ± 11.44	0.037

by χ^2-test & independent two sided t-test.
*QCA data were available in 179 lesions: 135 patent cases and 44 restenosis cases.
DS, diameter stenosis; MLD, minimal luminal diameter; RD, reference diameter; NS, not significant.

DD vs. II patients and 0.608 (95% CI, 0.244 - 1.511, p=0.284) for DD vs. II/ID patients.

However, the follow up MLD was significantly smaller in DD type than in II + ID type. Distribution of the ACE genotypes was compared in accordance with the follow-up MLD. Prevalence of the DD genotype was more frequent in lesions with MLD < 2 mm than in those with MLD > 2 mm (35.8% vs. 13.2%, χ^2-test, $p < 0.05$) (Fig. 2 and Table 4).

In the patent group, the distributions of TT, CT, and CC genotypes were 45.3%, 45.3%, and 9.4%, and in the restenosis group they were 41.2%, 51.5%, and 7.4%, respectively (Table 3). No significant differences were found in genotype distributions between the two groups. Binary multiple logistic regression analysis showed that the adjusted odds ratio was 0.391 (95% CI, 0.074 - 2.078, p=0.271) for CC vs. CT/TT patients and 1.191 (95% CI, 0.506 - 2.802, p=0.689) for CC/CT vs. TT patients.

In the patent group, the distributions of MM, MT, and TT genotypes were 16.4%, 30.2%, and 53.4%, and in the restenosis group they were 16.2%, 30.9%, and 52.9%, respectively (Table 3). The difference between the two groups was not

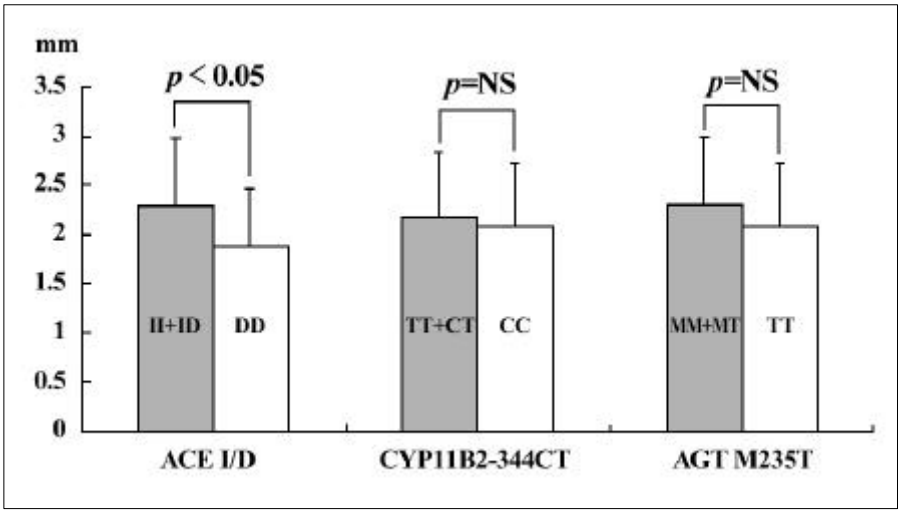

Fig. 2. Comparison of MLD at follow up angiography. NS, not significant.

Table 3. Comparison of Genotypes between the Patent and Restenosis Groups

	Patent group	Restenosis group	p value
ACE I/D			
II	64 (69.6%)	28 (30.4%)	
ID	94 (79.7%)	24 (20.3%)	0.238
DD	33 (73.3%)	12 (26.7%)	
CYP11B2			
TT	87 (84.3%)	28 (15.7%)	
CT	87 (71.3%)	35 (28.7%)	0.66
CC	18 (78.3%)	5 (21.7%)	
AGT M235T			
MM	31 (73.8%)	11 (26.2%)	
MT	57 (73.1%)	21 (26.9%)	0.994
TT	101 (73.7%)	36 (26.3%)	

by χ^2-test.

Table 4. Comparison of QCA Data According to Genotypes before and Immediately after Stenting

ACE I/D	II + ID (n=144)	DD (n=24)	*p* value
RD (mm)	3.2 ± 0.62	3.06 ± 0.51	0.308
Lesion length (mm)	17.71 ± 8.51	16.3 ± 7.55	0.451
MLD (mm)			
Before stenting	0.77 ± 0.52	0.96 ± 0.56	0.102
After stenting	3.11 ± 0.59	3.15 ± 0.38	0.672
F/U	2.29 ± 1.28	1.88 ± 0.87	0.044*
DS at F/U (%)	31.49 ± 24.94	39.36 ± 24.46	0.1
Late lumen loss (mm)	0.95 ± 0.81	1.22 ± 0.87	0.136
Rate of MLD < 2 mm†	33.1%	53.1%	0.033*
	TT + CT (n=155)	CC (n=17)	
RD (mm)	3.18 ± 0.61	3.19 ± 0.51	0.966
Lesion length (mm)	17.9 ± 8.76	16.31 ± 5.79	0.481
MLD (mm)			
Before stenting	0.81 ± 0.53	0.8 ± 0.55	0.924
After stenting	3.15 ± 0.58	3.23 ± 0.53	0.507
F/U	2.18 ± 0.94	2.08 ± 0.85	0.662
DS at F/U (%)	33.1 ± 25.36	34.23 ± 23.42	0.853
Late lumen loss (mm)	1 ± 0.85	1.03 ± 0.57	0.895
Rate of MLD < 2 mm†	35.5%	2.9%	0.157
	MM + MT (n=80)	TT (n=94)	
RD (mm)	3.17 ± 0.57	3.18 ± 0.64	0.909
Lesion length (mm)	17.91 ± 7.76	17.3 ± 9.04	0.654
MLD (mm)			
Before stenting	0.84 ± 0.52	0.78 ± 0.53	0.44
After stenting	3.15 ± 0.54	3.1 ± 0.61	0.665
F/U	2.3 ± 0.93	2.08 ± 0.93	0.125
DS at F/U (%)	30.55 ± 23.97	35.1 ± 26.10	0.219
Late lumen loss (mm)	0.92 ± 0.78	1.06 ± 0.88	0.317
Rate of MLD < 2 mm†	35.1%	37.5%	0.736

by independent 2-sided t-test, † χ^2-test, *$p<0.05$.
Rate of MLD < 2 mm was measured at follow up angiography.
F/U, follow up.

significant. Binary multiple logistic regression analysis showed that the adjusted odds ratio was 1.567 (95% CI, 0.681-3.605, p=0.291) for TT vs. MT/MM patients and 1.722 (95% CI, 0.498- 5.958, p=0.391) for TT/MT vs. MM patients. To exclude effects of ACE inhibitors, analysis of phenotypes of the three gene polymorphisms was done in the patients without administration of ACE inhibitors. There were no significant differences in restenosis rate between genotypes in any of the three gene polymorphisms (Table 5).

Synergistic effect of the three gene polymorphisms

To elucidate the synergistic effects of the gene polymorphisms, patients with ACE DD, AGT M235 TT, and CYP11B2-344 CC were selected

Table 5. Comparison of Genotypes between the Patent and Restenosis Groups in the Patients without Administration of ACE Inhibitors

	Patent group	Restenosis group	*p*-value
ACE I/D			
II	43 (71.7%)	17 (28.3%)	
ID	64 (81.0%)	24 (19.0%)	0.423
DD	24 (75.0%)	8 (25.0%)	
CYP11B2			
TT	62 (81.6%)	14 (18.4%)	
CT	56 (70.0%)	24 (30.0%)	0.235
CC	14 (77.8%)	4 (22.2%)	
AGT M235T			
MM	21 (75.0%)	7 (25.0%)	
MT	38 (70.4%)	16 (29.6%)	0.46
TT	70 (79.5%)	18 (20.5%)	

by χ^2-test.

and compared with the other patients. No significant difference was apparent in the prevalence of binary in-stent restenosis between the two groups. Following adjustment for age, sex, DM, hypertension, smoking, lesion length, and post procedural MLD using multiple binary logistic regression, the odds ratio (OR) was 0.988 (95% CI, 0.080 - 12.131, p=0.992). Patients with at least 1 mutation allele in each gene were also selected, and compared with the other subjects to determine the synergistic effects of mutation alleles in the three genes. Similarly, no significant difference in the prevalence of binary in-stent restenosis was found. Multiple logistic regression analysis also showed no contribution of homozygous or heterozygous mutation to in-stent restenosis (OR=0.978, 95% CI 0.387 - 2.469, p=0.962). Another method was adopted to investigate the synergistic effect. Wild type was scored 0, heterozygous and homozygous mutation types were scored 1, and the sum of each gene's score was calculated. No significant difference was found in the prevalence of binary in-stent restenosis and follow-up MLD according to the score, nor was there when wild type and heterozygous mutation type were scored 0 and homozygous mutation type was scored 1.

DISCUSSION

RAAS plays an important role in the development of hypertensive end-organ damage, not only as a stasis but also as a modulator of vascular tone and structure, and of cardiac and renal tissue remodeling.[29] These effects are primarily mediated by the final product of its enzymatic cascade, that is, angiotensin II and aldosterone, which have been shown to promote cellular growth and interstitial matrix deposition by multiple mechanisms.[30] Previous studies have shown that the ACE, AGT, and CYP11B2 gene polymorphisms increase the plasma and tissue level of angiotensin II and aldosterone.

Restenosis after balloon PTCA is a complex and only partially understood phenomenon. Early events after balloon injury include elastic recoil, platelet deposition, and thrombus formation, which are followed by subsequent smooth muscle cell proliferation and matrix formation.[31] But in coronary stenting, stents inhibit negative arterial remodeling (a decrease in arterial or external elastic membrane cross-sectional area), with neointimal hyperplasia being predominantly responsible for in-stent restenosis. Previous animal studies showed that RAAS is related to neointimal

hyperplasia and that neointimal proliferation was limited with the administration of ACE inhibitor.[32,33] These results suggested that angiotensin II or aldosterone is associated with smooth muscle cell overgrowth, and therefore that the polymorphisms of these genes might promote the proliferation of cardiovascular tissue and restenosis of coronary stenting.[34] Our study was designed to elucidate the relationship between genetic RAAS polymorphisms, namely ACE I/D, AGT M235T, and CYP11B2, and restenosis after coronary stenting.

In the present study, no significant relation was found between the ACE I/D polymorphism and the classic binary restenosis (follow up diameter stenosis [DS] >50%) rate after stenting. However, on analyzing QCA data, follow-up MLD was found to be significantly greater in the II+ID genotype than in the DD genotype ($p<0.05$). Although there was no statistical significance ($p=0.1$), DS and late lumen loss were greater in the DD genotype. In a study with IVUS and stress myocardial perfusion imaging, Nishihiko et al. reported that lesion cross-sectional area (CSA) $\leq$ 4mm^2 was a simple and highly accurate criterion for significant coronary narrowing.[35] Abizaid et al. reported that IVUS MLD $\geq$ 2 mm and lesion CSA $\geq$ 4 mm^2 had a diagnostic accuracy of 89% in identifying CFR $\geq$ 2.23 Therefore we divided the lesions into two groups of MLD $\geq$ 2 mm and MLD < 2 mm, and compared their genotypic distributions. In the lesions with follow-up MLD of < 2 mm, the frequency of the DD genotype was significantly higher than that of the II + ID genotypes (χ^2-test, $p<0.05$). This suggested that the progress of restenosis was more prominent in the DD genotype, that the ACE I/D polymorphism influenced the progress of restenosis in a recessive manner, and that the ACE DD genotype was associated with the development of clinically significant in-stent restenosis.

Previous studies showed that the ACE I/D polymorphism was not associated with restenosis after conventional balloon angioplasty.[36,37] Moreover, two randomized trials failed to demonstrate any beneficial effect of ACE inhibition on the occurrence of angiographic restenosis after balloon angioplasty.[38,39] However, multiple results on the classic binary restenosis (DS > 50%) rate after coronary stenting were inconsistent. In 146 patients, Amant et al. found an association between the presence of the D allele and angiographic restenosis that was compatible with the assumption of the co dominant effect for this allele.[5] Ribichini reported a similar result in 176 patients that did not receive ACE inhibitors, and also reported that the D allele had a co dominant effect on the phenotype (ACE level).[6] Our study showed a positive correlation between in-stent restenosis and ACE genotypes, but the correlation was weak compared to the two previously mentioned studies. This may be due to the lower frequency of D allele in the Korean population. Hong et al. reported that the frequency of the D-allele was lower in Koreans than in Caucasian (0.43 vs. 0.53), and their result was compatible with that of our study.[4] In particular, the frequencies of the DD genotype, the significant prognostic factor, were 18.2% and 35.2% in our study and the study of Ribichini et al., respectively. On the other hand, in a large subject group (n=1,850), Koch et al. showed that the ACE I/D polymorphism did not have an effect on in-stent restenosis.[9] The exact reason for the differences between the results of these previous studies remains unknown, but the complexity of the pathogenesis of neointimal hyperplasia and differences in study population size and race could be possible reasons for the wide spectrum of results.

To our knowledge, there has been no other research aimed at evaluating the association between AGT M235T and CYP11B2 gene polymorphisms and prognosis after coronary stenting. There have been many studies on the relation between these gene polymorphisms and LVH, arterial hypertension, and myocardial infarction. In 175 Chinese with hypertension, Jeng et al. reported that the TT genotype of the AGT gene could be considered a risk factor for the development of cardiac hypertrophy.[28] They suggested that the increased activity of angiotensin II in the TT genotype plays a role in hypertrophy. In a case-control study of 301 white male subjects, Winkelmann et al. reported that a stepwise increase in AGT plasma levels was seen in the presence of 1 or 2 T235 alleles and that a significant relationship existed between the AGT M235T variant and the cardiovascular disease pheno-

types, including diastolic hypertension, coronary artery disease, and myocardial infarction.[40] On the other hand, Yamada et al. reported that ACE I/D and AGT M235T polymorphisms were not related to hypertrophic and dilated cardiomyopathy in the Japanese population.[27] Our study on in-stent restenosis also showed a negative correlation, suggesting that the AGT M235T polymorphism does not have an effect on restenosis.

Kupari et al. reported that CYP11B2-344C was associated with the elevation of aldosterone in plasma and was strongly related to left ventricular size and mass in young adults without clinical heart disease.[41] Hautanen et al. reported that smoking and dyslipidemia were more potent risk factors for nonfatal myocardial infarction (MI) in males with CYP11B2 -344C.[42] However, Patel et al. reported that the CYP11B2-344 promoter region polymorphism did not significantly influence the risk of MI either directly or by interaction with other risk factors.[43] And Schunkert et al. reported that neither renin nor aldosterone level was affected by -344C/T allele status.[12] In our study CYP11B2 -344C did not show any influence upon the risk of restenosis after coronary stenting, regardless of adjustment for other risk factors such as age, sex, DM, hypertension, obesity, lesion length, and reference vessel diameter.

In this study, no synergistic effects, in either a recessive or dominant manner, were found for ACE, CYP11B2, and AGT gene polymorphisms upon in-stent restenosis. The negative correlation between CYP11B2 and AGTM235T polymorphisms and in-stent restenosis could be due to the discrepancy in the tissue and plasma levels of angiotensinogen and aldosterone. The complexity of pathogenesis of in-stent restenosis may also have affected the results of this study.

The present study had several limitations. Especially, the plasma activities of gene products, such as aldosterone, ACE, angiotensin II and angiotensinogen, were not measured in our study. Therefore it remained undetermined whether or not the negative correlations of the genetic polymorphisms are due to the absence of correlation with the concentrations of aldosterone, ACE, and angiotensin II.

In conclusion, the ACE I/D polymorphism may promote the progress of in-stent restenosis after coronary stenting in an autosomal recessive manner. MLD of less than 2 mm was reported to have clinical significance, and in such lesions the frequency of the DD genotype was higher than that of the other genotypes examined. However, its role as a risk factor of the classic binary restenosis could not be confirmed on the basis of our results. CYP11B2 -344C and AGT M235T polymorphisms were not risk factors of binary restenosis directly or via interaction with other risk factors, and no synergistic effect between these gene polymorphisms and in-stent restenosis was apparent.

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