The effects of the renin-angiotensin-aldosterone system gene polymorphism on coronary in-stent restenosis

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=Abstracts=

The effect of the renin-angiotensin-aldosterone system (RAAS) gene polymorphism on coronary in-stent restenosis

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In-stent restenosis is one of the most important prognostic factors in patients with coronary artery obstructive disease after intracoronary stent implantation. The intima proliferative model is recognized as a leading mechanistic theory of restenosis after stenting. The over excretion of angiotensin II and aldosterone, due to abnormality of the renin-angiotensin-aldosterone system (RAAS), is reported to be a possible cause of cardiovascular tissue proliferation, left ventricular hypertrophy, and hypertension. As polymorphisms of angiotensin I-converting enzyme (ACE), angiotensinogen (AGT), and aldosterone synthase (CYP11B2) are related to the high activity of each hormone, therefore, there are possibilities that the genes may affect the prognosis of in-stent restenosis. We analyzed genotypes of 241 patients who underwent intracoronary stent implantation and follow-up coronary angiography from year 1998 to 1999. And the outcome of 272 stents in these patients, past histories, and characteristics of the lesions were reviewed. The results were as follows;

1. The average age of patent and restenotic patients was 59.7±9.8 and 59.2±10.2 years old respectively. No significant difference in terms of sex was found between the two groups.
2. The prevalence of diabetes mellitus was significantly higher in the restenosis group by the χ^2- test ($p<0.05$).

3. The results of QCA (Quantitative Computer-assisted Analysis) revealed that the risk of in-stent restenosis increased with the lesion length and was inversely proportional to the lesion diameter.

4. Frequencies of II, ID, and DD genotypes were 81(33.6 %), 105(43.6 %), and 41(17.0 %) respectively in terms of the distribution of I/D polymorphism of ACE gene in the study population. Frequencies of TT, CT, and CC genotypes were 109(42.3 %), 109(42.3 %), and 21(8.7 %) respectively in the distribution of –344C polymorphism of CYP11B2 gene in the study population. Frequencies of MM, MT, and TT genotypes were 38(15.8 %), 72(29.9 %), and 119(49.4 %) respectively in terms of the distribution of M235T polymorphism of AGT gene in the study population.

5. In the case of ACE DD genotype, follow-up MLD (minimal luminal diameter) was significantly smaller than in those of II or ID genotypes by independent sample t-test ($p<0.05$). Also, in lesion with follow-up MLD < 2 mm, the ACE DD genotype was more frequent than the others.

6. There was no significant correlation between CYP11B2-344C and AGT M235T polymorphism and restenosis after stenting (χ^2- test, Multiple logistic regression test, one-way ANOVA test).

7. Synergistic effects of ACE, CYP11B2, and AGT gene polymorphisms upon in-stent restenosis were not found in either a recessive or dominant manner.

8. Although DM was one of the independent risk factors for in-stent restenosis, ACE, CYP11B2, and AGT gene polymorphism had no significant impact on in-stent restenosis in DM patients.

As described above, ACE I/D polymorphism promoted the progress of in-stent restenosis and was of clinical significance, but the other potential variables examined did not correlate with in-stent restenosis.

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

The effects of the renin-angiotensin-aldosterone system gene polymorphism on coronary in-stent restenosis

I. Introduction

Since percutaneous balloon angioplasty and stent implantation have been used for the treatment of coronary artery disease, in-stent restenosis has been recognized as the most important obstacle to favorable prognosis. Constrictive remodeling with adventitial scarring is usually considered to be the most important mechanism of restenosis following percutaneous balloon angioplasty. However, after stenting the mechanism of restenosis almost exclusively involves neointimal hyperplasia.[1]

Genes involved to rennin-angiotensin-aldosterone system (RAAS) were investigated as possible risk factors of cardiovascular and renal disease. Angiotensin I converting enzyme (ACE) is a core factor for the production of angiotensin II and degradation of bradykinin. High activities of ACE increase the vessel wall thickness and cause thrombosis, constriction, and the proliferation of smooth muscle cells.[2] The ACE gene has now been cloned, and an insertion (I)-deletion (D) polymorphism in intron 16 has been identified.[3] 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 LVH[4] and myocardial infarction.[5] An association between the presence of the D allele and angiographic in-stent restenosis has been reported in some previous studies.[6,7] Although studies described above have shown a positive relation between polymorphism and restenosis, the relationship remains controversial.[8]

Aldosterone is a mineralocorticoid hormone, which via renal

actions, controls sodium balance and intravascular volume, and thus helps to regulate blood pressure.[9] In addition, aldosterone may have several direct actions on the heart, including the development of cardiac hypertrophy and fibrosis by stimulating cardiac collagen synthesis and fibroblast proliferation.[10,11] Variations in the CYP11B2 gene may influence the activity of aldosterone.[12] This is particularly true of the cytosine/thymidine(C/T) exchange at position –344 in the regulatory region of the aldosterone synthase (CYP11B2) gene, which is associated with the plasma and urinary levels of aldosterone.[13,14] The CYP11B2 gene polymorphism was reported to be associated with the enlargement and disturbed filling of LV and hypertension. But the association with prognosis after stenting has not been the subject of study.

A molecular variant (M235T) of the angiotensinogen (AGT) gene exists in exon 2, consists 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.[15,16] Recent studies have shown that the AGT M235T polymorphism is linked with hypertension, coronary atherosclerosis, and LVH via cellular proliferation.[15,17,18] The M235T polymorphism may induce restenosis after stenting via the increased activity of angiotensin II, but no study has been undertaken to date.

The aim of this study is to explore whether gene polymorphisms of the RAAS influence the phenotypic expression of restenosis after stenting in a sample of Korean patients with coronary stenting.

II. Materials and Methods

1. Materials

This study included 241 consecutive patients with coronary artery disease that underwent coronary artery stent implantation between 1998 and 1999 at the Yonsei cardiovascular center, Yonsei University, Seoul, Korea. Coronary stenting was performed on 272 lesions in these 241 patients and follow-up angiography and blood sampling for genetic analysis were performed in all subjects. The patients that underwent stent implantation within 3 days of acute myocardial infarction were excluded to rule out the influence of thrombus. Information about age, sex, body mass index (BMI), angiographic diagnosis, serum lipid profile, and past history was obtained from medical records.

2. 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 (ANCOR version 2.0, Siemens). Angiography was routinely performed in at least 2 projections. These projections were recorded in our database, and follow-up angiography was 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 restenosis, a categorical approach with the classic criteria of >50% diameter stenosis during follow-up angiography was adopted.[19,20] Abizaid et al. reported that intravascular ultrasonography (IVUS) MLD $\geq$ 2mm had a diagnostic accuracy of 89% in terms of identifying coronary flow reserve (CFR) $\geq$ 2.[21]

Therefore lesions were divided into two groups, namely, less than 2.0 mm and equal to or greater than 2.0 mm for comparison with the genotypic distribution.

3. Genotyping

1) ACE gene polymorphism

Genomic DNA was extracted from 200 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. 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 Polymerase (AmpliTaq Gold™, Roche Molecular System, Inc., Branchburg, New Jersey, USA). Amplification was performed with a 9600 Perkin Elmer Thermal Cycler. Samples were denatured for 10 minutes at 92°C and then cycled 37 times through as follows: 30 seconds at 92°C, 30 seconds at 67°C, and 1 minute at 72°C. The last extension was performed for 5 minutes at 72°C. 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) was detected as a 190bp band. (Fig. 1) Insertion specific PCR (ISP) was performed to prevent the underestimation of heterozygotes and the 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'), with identical PCR conditions. (Fig. 2)

2) 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. PCR was performed in a final volume of 20 μL that contained 50 ng of genomic DNA, 10 pmol of each primer, 0.0625 mM dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), and 0.4 U *AmpliTaq* DNA Polymerase (AmpliTaq Gold™, Roche Molecular System, Inc., Branchburg, New Jersey, USA). After an initial denaturation at 95°C for 10 minutes, samples were cycled 35 times as follows; 1 minute at 95°C, 1 minute at 67°C, and 2 minutes at 72°C. Final extension was then performed for 5 minutes at 72°C. The PCR product was restricted with 5 U of the restriction endonuclease HaeIII (Takara™) over 2 hours at 37°C, and the final product was electrophoresed in 2% agarose gel and visualized directly by ethidium bromide staining. The –344T allele lacks of a HaeIII site that is present in the –344C allele and gives rise to a fragment of 273bp rather than 202bp. (Fig. 3)

3) 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°C for 5 minutes, samples were cycled 38 times through the following steps; 15 seconds at 94°C, 45 seconds at 60°C, and 45 seconds at 72 °C. Extension was performed for 10 minutes at 72°C. The PCR product was incubated with Tth111/AspI (Takara™) at 67°C 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 Tth111/AspI 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. 4)

4. 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 > 2 groups. Independent association between the genotypes of each gene and outcome was assessed after adjusting for other potential confounding factors by multiple logistic regression analysis.

To find out recessive effect for the mutant allele, the patients were divided into group A and B, group A contained the genotypes of II+ID (ACE I/D), TT+CT (CYP11B2 –344C), and MM+MT (AGT M235T) and group B contained the genotypes of DD (ACE I/D), CC (CYP11B2-344C), and TT (AGT M235T). To find out dominant effect for the mutant allele, the patients were divided into group A and B, group A contained the genotypes of DD (ACE I/D), CC (CYP11B2 –344C), and TT (AGT M235T) and group B contained the other genotypes.

III. Results

1. Characteristics of the subjects

The mean age of the patients were 59.4 ± 10.2 years and 74.3% were men. Mean ages of the patent and restenosis groups were 59.7 ± 9.8 and 59.2 ± 10.2, respectively, which was not significantly different, and neither were sex, smoking history, serum level of lipid (total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride), BMI, or the prevalence of hypertension. However, the prevalence of diabetes mellitus was significantly higher in the restenosis group ($p<0.05$). (Table 1)

Table 1. Clinical characteristics of the patients

	Patent group (n=167)	Restenosis group (n=74)	p value
Age (years)	59.7 ± 9.8	59.2 ± 10.2	NS
Male (%)	116 (77.3%)	36 (72.0%)	NS
Past History			
DM	47 (24.1%)	28 (36.4%)	< 0.05
Hypertension	96 (49.2%)	37 (48.7%)	NS
Smoking (PYS)	33.1 ± 20.2	32.5 ± 16.3	NS
Total Chol (mg/dl)	199.0 ± 40.8	199.7 ± 39.1	NS
HDL Chol (mg/dl)	45.2 ± 25.7	45.6 ± 30.3	NS
LDL Chol (mg/dl)	126 ± 59.5	133.8 ± 83.9	NS
TG (mg/dl)	158.8 ± 98.9	147.4 ± 76.8	NS
Lp (a) (mg/dl)	35.9 ± 78.2	37.3 ± 27.2	NS
Fibrinogen (mg/dl)	391.0 ± 154.4	384.7 ± 118.1	NS

NS; not significant PYS; pack years Chol; cholesterol TG; triglyceride

2. Angiographic data

Of 271 stents, the patent and restenosis groups represented 71.7% and 28.3% respectively. The prognosis of stenting was evaluated according to the characteristics of lesions. In-stent restenosis was more frequent in lesions over 20 mm ($p<0.01$). Restenosis was more frequent in angled lesion (>45°) but this was not statistically significant, and neither did the presence of calcification and thrombus had a significant influence on the prognosis of stenting (Table 2). QCA revealed that the proximal reference and distal reference diameters were significantly greater in the patent group than the restenosis group ($p=0.011$ and <0.01 respectively). No significant difference was observed in terms of pre-stenting MLD, percent diameter stenosis, or post-stenting residual stenosis between the two groups. However, lesion length was significantly greater in the restenosis group than the patent group (Table 3).

Table 2. Comparison of morphological characteristics of stent insertion site between the patent and restenosis groups

	Patent group	Restenosis group	p value
Lesion length			
Discrete (<10mm)	62 (33.3%)	19 (25.7%)	
Tubular (10-20mm)	78 (41.9%)	21 (28.4%)	<0.01
Diffuse (>20mm)	46 (24.7%)	34 (45.9%)	
Lesion angulations			
< 45°	175 (92.6%)	64 (87.7%)	
≥ 45° and < 90°	14 (7.4%)	7 (9.6%)	0.06
≥ 90°	0 (0%)	2 (2.7%)	
Calcification			
No calcification	179 (94.2%)	67 (90.5%)	
Calcification	11 (5.8%)	7 (9.5%)	NS
Patency			
Patent	170 (89.9%)	60 (82.2%)	
Total occlusion (< 3months)	15 (7.9%)	10 (13.7%)	NS
Total occlusion (> 3months)	4 (3.2%)	3 (4.1%)	
Thrombus			
No thrombus	160 (85.6%)	65 (89%)	
Thrombus	27 (14.4%)	8 (11%)	NS

by χ^2-test NS ; not significant

Table 3. Comparison of QCA data between the patent and restenosis groups

	Patent group (n = 131)	Restenosis group (n = 49)	p value
QCA Data			
p-Ref.Diameter (mm)	3.39 ± 0.57	3.14 ± 0.61	0.011
d-Ref.Diameter (mm)	3.12 ± 0.64	2.80 ± 0.77	<0.01
Pre MLD (mm)	0.85 ± 0.6	1.12 ± 2.67	NS
Pre DS (%)	74.86 ± 15.2	74.18 ± 13.69	NS
Post MLD (mm)	3.43 ± 2.02	3.48 ± 3.58	NS
Post DS (%)	1.9 ± 8.6	1.19 ± 15.27	NS
Lesion length (mm)	16.8 ± 7.4	19.8 ± 10.7	<0.05

by independent two sided t-test NS ; not significant DS; diameter stenosis MLD; minimal luminal diameter p-Ref.Diameter; proximal reference diameter d-Ref.Diameter; distal reference diameter

3. Genotypes

1) ACE genotyping

The distribution of II, DI, and DD genotypes were 33.6% (n=81), 43.6% (n=105), and 17.0% (n=41) respectively (Fig. 1, 2). The frequencies of these genotypes were compatible with the Hardy-Weinberg distribution and close to values reported in other studies on Koreans.[22] In the patent group the distribution of II, DI, and DD genotypes were 34.4%, 48.4%, and 17.2%, and in the restenosis group they were 30.4%, 40.6%, and 18.8% respectively (Table 4). There were no significant differences in genotypes between the two groups. Using multiple logistic regression analysis, adjustment was made for age, sex, arterial hypertension, DM, current smoking habit, vessel location, multivessel disease, lesion length, vessel diameter, BMI, and post-procedural MLD. The adjusted odds ratio was 1.561(95% confidence

interval (CI), 0.602-4.046, not significant) for ID/DD vs. II patients and 1.32(95% CI, 0.355 – 4.904, not significant) for DD vs. II/ID patients.

Follow up MLD was significantly larger in II + ID type than in DD type. Distribution of the ACE genotypes was compared in accord with the follow-up MLD. In lesions with MLD $\geq$ 2mm, the distribution of II, DI, and DD genotypes were 37(32.5%), 62(54.4%), and 15(13.2%) respectively and for an MLD < 2 mm these were 26(39.4%), 23(34.8%), and 17(25.8%) (χ^2-test, $p < 0.05$). In lesions with MLD $\geq$ 2 mm, II + ID prevalence was greater than in that of MLD < 2mm (86.8% vs. 74.2%, χ^2-test, $p<0.05$) (Table 5, 6). The frequency of genotype was related to follow-up MLD, but not with binary restenosis.

In patients with DM, DD type was more frequently associated with restenosis than other types (32% vs. 17.4%), but the difference was statistically insignificant. In obese (BMI>27 kg/m^2) or smoking patients with DD type, the incidence of restenosis was similar to those with other types (Table 7).

2) CYP11B2 genotyping

The distribution of TT, CT, and CC genotypes were 42.3% (n=109), 42.3% (n=109), and 8.7% (n=21), respectively (Fig 3). The frequencies of these genotypes were compatible with the Hardy-Weinberg distribution and close to values reported in Japanese studies.[23] In the patent group the distribution of TT, CT, and CC genotypes were 45.5%, 44.9%, and 9.6% and in the restenosis group were 41.1%, 52.1%, and 6.8% respectively (Table 4). No significant differences were found in genotypes of the two groups. Using multiple logistic regression analysis, we adjusted for age, sex, arterial hypertension, diabetes mellitus, current smoking habit, vessel location, multivessel disease, lesion length, vessel diameter, BMI, and post procedural MLD. The adjusted odds ratio was 0.696(95% CI, 0.285-1.700, not significant) for CC vs. CT/TT patients and 0.857(95% CI, 0.198 – 3.702, not significant) for CC/CT vs. TT

patients.

Table 4. Comparison of genotypes between the patent and restenosis groups

	Patent group	Restenosis group	p-value
ACE I/D			
II	64 (34.4%)	28 (30.4%)	
ID	90 (48.4%)	28 (40.6%)	NS
DD	32 (17.2%)	13 (18.8%)	
CYP11B2			
TT	85 (45.5%)	30 (41.1%)	
CT	84 (44.9%)	38 (52.1%)	NS
CC	18 (9.6%)	5 (6.8%)	
AGT M235T			
MM	30 (16.2%)	12 (16.7%)	
MT	53 (28.6%)	25 (34.7%)	NS
TT	102 (55.0%)	35 (48.6%)	

by χ^2-test NS ; not significant

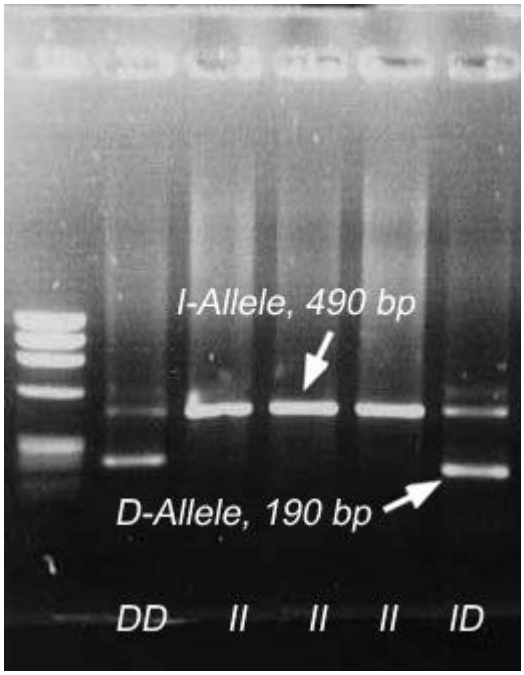

Fig. 1 Genotyping of ACE gene I/D polymorphism

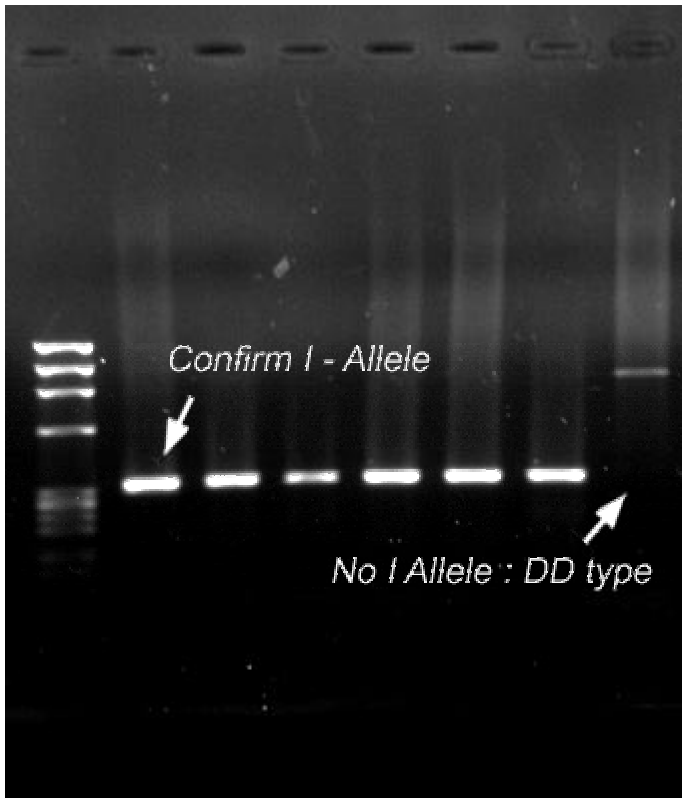

Fig.2 Confirmation of I allele by I specific PCR (ISP)

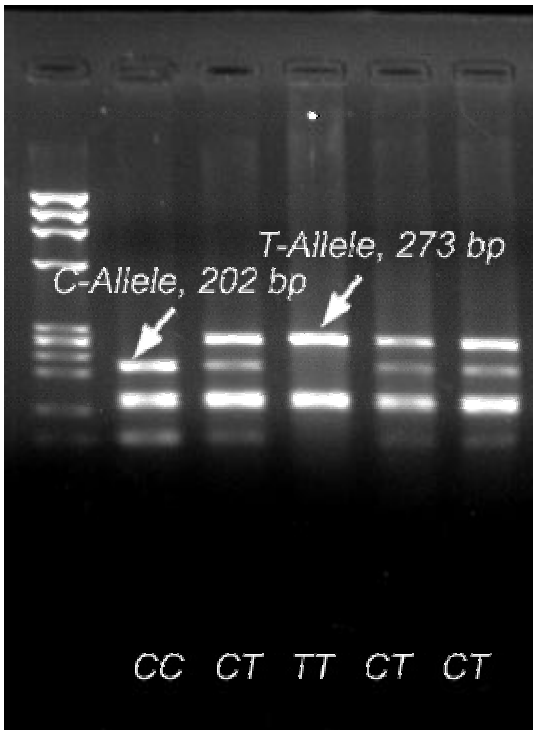

Fig. 3 Genotyping of the CYP11B2 gene polymorphism

3) AGT genotyping

The frequency of each genotype was MM: 15.8%(n=38), MT: 29.9%(n=72), and TT: 49.4%(n=119) (Fig. 4), which are compatible with the Hardy-Weinberg distribution and close to the values reported in other studies with Japanese and Chinese.[24-26] In the patent group, the distribution of MM, MT, and TT genotypes were 17.4%, 39.1%, and 43.5% respectively and in the restenosis group were 18.5%, 37.0%, and 44.0% (Table 4). The difference between the two groups was not significant. Using multiple logistic regression analysis, adjustment was made for age, sex, arterial hypertension, diabetes mellitus, current smoking habit, vessel location, multivessel disease, lesion length, vessel diameter, body mass index (BMI), and post procedural MLD. The adjusted odds ratio was 0.547(95% CI, 0.15-1.97, not significant) for TT vs. MT/MM patients and 1.450(95% CI, 0.624 – 3.337, not significant) for TT/MT vs. MM patients.

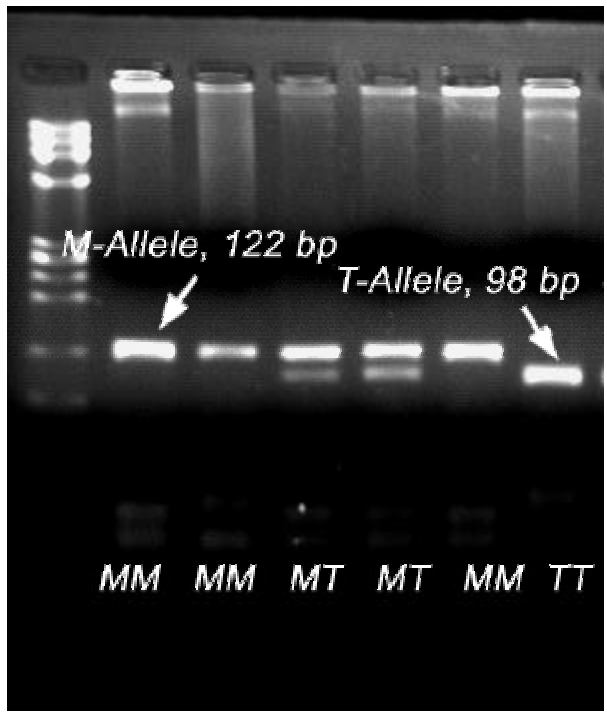

Fig.4 Genotyping of AGT gene M235T polymorphism

Table 5. Comparison of genotypes between patent and restenosis group in DM patients

	Patent group	Restenosis group	p value
ACE I/D			
II	17 (37%)	6 (24.0%)	
ID	21 (45.7%)	11 (44.0%)	NS
DD	8 (17.4%)	8 (32.0%)	
CYP11B2			
TT	20 (43.5%)	15 (55.6%)	
CT	19 (41.3%)	9 (33.3%)	NS
CC	7 (15.2%)	3 (11.1%)	
AGT M235T			
MM	8 (17.4%)	5 (18.5%)	
MT	18 (39.1%)	10 (37.0%)	NS
TT	20 (43.5%)	12 (44.0%)	

by χ^2-test NS ; not significant

Table 6. Comparison of QCA data according to genotypes before and immediately after stenting

ACE I/D	II + ID (n =156)	DD (n =33)	P value
Reference diameter (mm)	3.35 ± 0.60	3.14 ± 0.59	NS
Lesion length (mm)	17.71 ± 8.51	16.30 ± 7.55	NS
Before stenting			
MLD (mm)	0.77 ± 0.52	0.96 ± 0.56	NS
Diameter stenosis (%)	75.79 ± 14.61	70.18 ± 16.78	NS
After stenting			
MLD (mm)	3.11 ± 0.59	3.15 ± 0.38	NS
Diameter stenosis (%)	2.30 ± 11.40	1.81 ± 9.24	NS
	TT + CT (n =164)	CC (n =17)	
Reference diameter (mm)	3.33 ± 0.61	3.37 ± 0.55	NS
Lesion length (mm)	17.90 ± 8.76	16.31 ± 5.79	NS
Before stenting			
MLD (mm)	0.81 ± 0.53	0.80 ± 0.55	NS
Diameter stenosis (%)	74.60 ± 14.93	77.09 ± 15.60	NS
After stenting			
MLD (mm)	3.12 ± 0.58	3.23 ± 0.53	NS
Diameter stenosis (%)	2.26 ± 11.41	1.01 ± 7.26	NS
	MM + MT (n=156)	TT (n=33)	
Reference diameter (mm)	3.34 ± 0.59	3.14 ± 0.59	NS
Lesion length (mm)	17.71 ± 8.51	16.30 ± 7.55	NS
Before stenting			
MLD (mm)	0.84 ± 0.52	0.78 ± 0.53	NS
Diameter stenosis (%)	73.89 ± 14.78	75.78 ± 15.12	NS
After stenting			
MLD (mm)	3.15 ± 0.54	3.10 ± 0.61	NS
Diameter stenosis (%)	1.23 ± 10.58	2.95 ± 11.43	NS

by independent 2-sided t-test

NS; not significant

Table 7. Comparison of QCA data of follow-up coronary angiography

ACE I/D	II + ID (n=156)	DD (n=33)	P value
MLD (mm)	2.29 ± 1.28	1.88 ± 0.87	< 0.05
Diameter stenosis (%)	31.49 ± 24.94	39.36 ± 24.46	0.101
Late lumen loss (mm)	0.95 ± 0.81	1.22 ± 0.87	0.136
Restenosis rate (DS > 50%)	27.1%	28.9%	NS*
Rate of MLD < 2 mm	33.1%	53.1%	< 0.05*
CYP 11B2-344C/T	TT + CT (n=164)	CC (n =17)	
MLD (mm)	2.23 ± 1.27	2.08 ± 0.85	NS
Diameter stenosis (%)	33.10 ± 25.36	34.23 ± 23.42	NS
Late lumen loss (mm)	1.0 ± 0.85	1.03 ± 0.57	NS
Restenosis rate (DS > 50%)	29.1%	21.7%	NS*
Rate of MLD < 2 mm	35.5%	52.9%	NS*
AGT M235T	MM + MT (n=156)	TT (n=33)	
MLD (mm)	2.27 ± 0.92	2.17 ± 1.43	NS
Diameter stenosis (%)	30.55 ± 23.97	35.10 ± 26.10	NS
Late lumen loss (mm)	0.92 ± 0.77	1.06 ± 0.87	NS
Restenosis rate (DS >50%)	29.1%	21.7%	NS*
Rate of MLD < 2 mm	35.1%	37.5%	NS*

by independent 2-sided t-test

* χ^2 –test , NS ; not significant

4) Synergistic effect of the three gene polymorphisms

To elucidate the synergistic effects of the gene polymorphisms, patients with ACE D/D, AGT M235 T/T, and CYP11B2-344 C/C were selected and compared with the other patients. No significant difference was apparent in the prevalence of binary in-stent restenosis between the two groups. Adjusting age, sex, DM, hypertension, BMI, smoking,

lesion length, and reference diameter using multiple logistic regression, the odds ratio (OR) was 1.222(95% CI, 0.487 – 3.064, not significant). Patients with at least 1 mutation allele in each gene were also selected, and compared with the other subjects to determine synergistic effects of mutation alleles in 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=1.295, 95% CI 0.0191-18.431, not significant).

IV. Discussion

The 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.[27] 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.[28] Previous studies have shown that the ACE, the AGT, and the CYP11B2 gene polymorphism increase the plasma and tissue level of angiotensin II and aldosterone.

Restenosis after plain 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.[29] 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 with the administration of ACE inhibitor neointimal proliferation was limited.[30,31] These results suggested that angiotensin II or aldosterone are associated with smooth muscle cell overgrowth, and that polymorphism of these genes might promote the proliferation of cardiovascular tissue and restenosis of coronary stenting.[32] Our study was designed to evaluate 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 binary restenosis rate after stenting. But on analyzing QCA data, follow-up MLD was found significantly greater in the II+ID genotype than in the DD genotype ($p < 0.05$). Although

there was no statistical significance (p=0.1), follow up diameter stenosis (DS) and late lumen loss were greater in DD genotype. In study with IVUS and stress myocardial perfusion imaging, Nishihiko et al. reported that the lesion cross-sectional area (CSA) $\leq$ 4mm^2 was a simple and highly accurate criterion for significant coronary narrowing.[33] Abizaid et al. reported that IVUS MLD $\geq$ 2mm and lesion CSA $\geq$ 4 mm^2 had a diagnostic accuracy of 89% in identifying CFR $\geq$ 2.[21] 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 DD, that the ACE I/D polymorphism influenced the progress of restenosis in recessive manner, and that the ACE DD genotype had association 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.[34,35] Moreover, two randomized trials[36,37] failed to demonstrate any beneficial effect of ACE inhibition on the occurrence of angiographic restenosis after balloon angioplasty. However, multiple results on restenosis after coronary stenting were inconsistent. In 146 patients Amante 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. 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). However, in a much larger subject group (n=1850), Koch et al. showed a negative correlation.[8] These reciprocal results might be due to differences in the sizes of study populations. In the meta-analysis of Samani et al., an inverse correlation was shown between the study sample size and the magnitude of relative risk associated with I/D polymorphism.[38] Our

study also showed no correlation between the D-allele and the binary restenosis rate after coronary stenting. Because the size of our study population was near to that of Amante and Ribichini, our negative result could not be due to the size of the study. These differences might reflect the complexity of polygenic, multifactorial disease. Another possible cause was the lower frequency of the D-allele in Koreans in contrast to that in western people. Hong et al. reported that the frequency of the I-allele was higher in Koreans (0.57 vs. 0.47), and the result was compatible with our study. In particular, the frequency of the D/D type that was considered to be a significant prognostic factor in previous studies was 18.2% and 35.2% in our study and the study of Ribichini et al. respectively.

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. But there have been many studies on the relation between these gene polymorphisms and left ventricular hypertrophy (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. They suggested that this was due to the increased activity of angiotensin II in the TT genotype.[26] 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 allele and that significant relations existed between the AGT M235T variant and the cardiovascular disease phenotypes, including diastolic hypertension, coronary artery disease, and myocardial infarction.[39] On the other hand Yamada et al reported ACE I/D and AGT M235T polymorphisms were not related to hypertrophic and dilated cardiomyopathy in the Japanese population. Our study on in-stent restenosis also showed negative correlation. Interestingly, in the present study, the distribution of the

AGT genotype differed from that of western study and was similar to the results of the studies in Chinese and Japanese.[24,25]

CYP11B2-344C is associated with the elevation of aldosterone in plasma.[40] Kupari et al. reported that CYP11B2-344C was strongly related to left ventricular size and mass in young adults free of clinical heart disease.[40] Hautanen et al. reported that smoking and dyslipidemia were more potent risk factors for nonfatal MI in males in individuals with CYP11B2 –344C.[41] 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, and Schunkert et al. reported that neither renin nor aldosterone level was affected by –344C/T allele status.[11] 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, including, age, sex, DM, hypertension, obesity, lesion length, and reference vessel diameter.

In this study synergistic effects of ACE, CYP11B2, and AGT gene polymorphisms upon in-stent restenosis were not found in either a recessive or dominant manner.

Our present study has some limitations. QCA analysis of angiographic data was not performed for all subjects. In some subjects binary restenosis was determined by eye, which creates the possibility of error. Plasma activities of gene products, such as, aldosterone, ACE, and angiotensin were not measured, and subjects who were taking ACE inhibitors or angiotensin II receptor inhibitors were included. With taking ACE inhibitor, the effects of these gene polymorphisms might be obscured. In this study group classification were based on MLD=2mm in QCA, but a previous study reported that QCA MLD had a poor correlation with CRF than IVUS MLD (r=0.552 vs. 0.782).[21]

V. Conclusion

ACE I/D polymorphism may promote the progress of in-stent restenosis after coronary stenting in an autosomal recessive manner. An MLD of less than 2 mm was reported to have clinical significance, and in this lesion the frequency of the DD genotype was higher than the other genotypes examined. However, on the basis of our results, its role as a risk factor of binary restenosis was uncertain. CYP11B2 –344C and AGT M235T polymorphisms were not risk factor 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.

VI. References

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ABSTRACT (In KOREAN)

관동맥 스텐트 삽입술 후 재협착에 대한 rennin-angiotensin-aldosterone system 유전자 다형성의 영향

관동맥 폐쇄질환의 치료로 관동맥 스텐트 삽입술 후의 예후에 있어 가장 많은 영향을 미치는 것 중 하나가 재협착이다. 현재 스텐트 삽입술 후 재협착의 기전 중 가장 유력한 것은 증식성 모델이다. 레닌-안지오텐신-알도스테론 시스템의 이상에 의한 알도스테론 및 안지오텐신 II의 과분비는 심혈관계 조직의 과증식을 유발하여 좌심실 종대 및 고혈압과 관련이 있는 것으로 보고되고 있으며, ACE 유전자, CYP11B2 유전자, AGT 유전자의 다형성은 이러한 호르몬의 과 분비를 유발하는 것으로 알려져 있다. 본 연구에서는 이러한 유전자들의 다형성 유전자형을 분석하여 스텐트 삽입술 후 재협착의 발생률과 관련이 있는지 밝혀냄으로써 유전적으로 재협착의 고위험군을 알아낼 수 있는지를 알아보았다. 대상 환자는 1998년부터 1999년까지 신촌 세브란스 병원에서 관동맥 질환을 진단 받고 경피적 스텐트 삽입술을 시행 받은 후 추적 관동맥 조영술을 시행 받았던 241명의 ACE, AGT, 그리고 CYP11B2 유전자 다형성의 유전자형을 분석하고 환자들의 272개 스텐트의 예후와 각각의 병변의 특성을 분석하여 다음과 같은 결과를 얻었다.

1.재협착군과 비재협착군의 평균 연령은 각각 59.7±9.8세, 59.2±10.2세로 차이가 없었으며 성별도 차이가 없었다.

2.고혈압의 유무, 갑년으로 계산한 흡연력 등도 각 군간에 차이가 없었으나 당뇨는 재협착군에서 유병률이 유의하게 높았다 ($p < 0.05$).

3.QCA(Quantitative Computer-assisted Analysis) 결과를 보면 근위부 표준 내경(proximal reference diameter)과 원위부 표준 내경(distal reference diameter)이 각각 유의도 0.011과 <0.01 수준으로 비 재협착 군에서 크게 나타났다.

4. ACE 유전자 I/D 다형성 분석에서 유전자형 빈도는 II/ID/DD 형이 각각 81 명 (33.6 %), 105 명 (43.6 %), 그리고 41 명 (17.0 %) 이었으며, CYP11B2 유전자 다형성에서 유전자형의 빈도는 TT, CT, CC형이 각각

109 명 (42.3 %), 109 명 (42.3 %), 그리고 21 명 (8.7 %) 이었다. 그리고 AGT유전자 M235T 다형성의 빈도는 MM이 38 명으로 15.8 %, MT형이 72 명으로 29.9 %, 그리고 TT형이 119 명으로 49.4 %이었다.
5. ACE 유전자 I/D 다형성 분석에서 DD 유전자의 경우 추적 관동맥 촬영에서 최소 혈관 내경이 II 나 ID 형에 비해 의미 있게 작았으며 ($p<0.05$), 임상적으로 의미를 가지는 것으로 보고된 최소 혈관 내경 2mm를 기준으로 나눈 경우에 2mm 이하의 환자 군에서 DD 유전자형을 가지는 경우가 나머지 보다 의미 있게 많았다 ($p<0.05$).
6. CYP11B2-344C 과 AGT M235T 유전자 다형성에서는 각각의 유전자형 간 재협착의 빈도에 있어 의미 있는 차이가 없었다 (χ^2-test, Multiple logistic regression test, one-way ANOVA test).
7. ACE, AGT, 그리고 CYP11B2 유전자 다형성 간에 상승작용이 있는지 알아보기 위해, 세 유전자에서 모두 mutation을 가지는 병변과 나머지 병변을 비교하였으나 통계학적인 차이는 없었다.

결론적으로 ACE I/D 유전자 다형성은 재협착의 진행에 영향을 주었다. 그러나 AGT M235T와 CYP11B2 유전자 다형성은 스텐트 재협착에 있어 직접적으로 또는 간접적으로 영향이 없었다. 그리고 위 3가지의 레닌-안지오텐신-알도스테론 시스템에 관여하는 유전자의 다형성은 서로 상승 작용이 없었다.

핵심되는 말 : 유전자 다형성, 스텐트 재협착
renin-angiotensin-aldosterone system