ANGIOTENSIN II TYPE I RECEPTOR AND ANGIOTENSINOGEN GENE POLYMORPHISMS IN PATIENTS WITH CORONARY ARTERY DISEASE

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Abstract: To analyze the participation of genetic factors in the onset and process of coronary arteriosclerosis, we investigated the distribution of both angiotensin II type I receptor (AT1R) and angiotensinogen (ATN) gene polymorphisms, and the relationship between genotype and clinical features in patients with coronary artery disease (CAD). We studied 139 CAD patients (CAD group : 93 males, 46 females, mean age of 62 y/o) consisting of 118 with myocardial infarction and 21 with angina pectoris. We selected 133 healthy volunteers without overt cardiac heart disease as control subjects. A1166C polymorphism of the AT1R gene (A→C transversion at position 1166 of AT1R gene) and T174M polymorphism of ATN gene (T→M transversion at position 174 of ATN gene) were determined by PCR of genomic DNA extracted from peripheral leukocytes and digestion with restriction enzyme. Patients were classified into one of three genotypes, AA homozygote, AC heterozygote and CC homozygote for AT1R, TT, TM and MM for ATN. The distribution of AT1R and ATN genotype did not differ between CAD patients and control subjects. Neither AT1R nor ATN genotype was correlated with the severity of coronary arteriosclerosis. In AT1R gene polymorphism, AC and CC genotype were significantly more frequent in patients with than in those without restenosis. In ATN gene polymorphism, TT genotype was also significantly more frequent in patients with than in those without restenosis. In conclusion, both AT1R and ATN gene polymorphism may participate in the process of restenosis after coronary angioplasty.
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Key words : angiotensin II type I receptor, angiotensinogen, gene polymorphism, coronary artery disease

INTRODUCTION

The pathogenesis of coronary artery disease (CAD) is multifactorial, while factors such as hypertension, hyperlipidemia, diabetes mellitus, smoking and obesity had been established as coronary risk factors. Furthermore, recent studies have suggested that genetic factors also play an important role in the genesis of arteriosclerosis in addition to environmental factors. Particularly, genes related to the renin-angiotensin system (RAS) have been reported to contribute to the development of CAD. In 1992, Cambien[1] reported that a homozygous deletion allele of the gene for angiotensin converting enzyme (ACE) was associated with an increased risk of myocardial infarction. Subsequently, many researchers investigated the relationships between ACE polymorphism and various heart diseases, such as hypertension[2], hypertrophic

cardiomyopathy[3], and the development of CAD[4]. Although there is no precise explanation why the ACE DD genotype is associated with the development of CAD, it is known that plasma concentration of ACE in patients with the ACE DD genotype is higher than in those with the other genotypes[5~6]

Another element of RAS, angiotensin II, has extensive effects on hypertrophy[7] and proliferation[8] of vascular smooth muscle. Considering that most of the actions of angiotensin II are mediated through the angiotensin II type I receptor (AT1R) and vascular injury induced angiotensinogen gene expression in the neointima of the injured vessel[9], the polymorphsm of ATN and AT1R may contribute to the development of CAD. In this study, to analyze the participation of genetic factors in the onset and process of coronary arteriosclerosis, we investigated the distribution of gene polymorphisms of angiotensin II type I receptor and of angiotensinogen (ATN) gene that regulate the production of angiotensin II, and the relationship between genotype and clinical features in patients with CAD.

PATIENTS AND METHODS

Study Subjects

We studied 139 CAD patients consisting of 118 cases of myocardial infarction and 21 cases of angina pectoris. All patients were admitted to Nara Medical University Hospital for further examination of abnormal findings on electrocardiograph, pericardial oppression and other symptoms. The diagnoses of myocardial infarction and angina pectoris were made by physical examination, electrocardiogram, and echocardiography, and the presence of CAD was confirmed by coronary angiography in 130 patients. CAD patients were divided into 2 groups according to the angiographical severity of coronary arteriosclerosis, as single-vessel disease (SVD) (n=52) and multi-vessel disease (MVD) (n=78). One hundred and seven patients who had undergone successful percutaneus transluminal coronary angioplasty (PTCA) received angiographical follow-up for less than six months. In this study, successful PTCA was defined as a visually assessed stenosis diameter of less than 50 % after angioplasty. We divided these patients into two groups : restenosis group and non-restenosis group. Restenosis after coronary angiography was defined as a more than 50 % loss of the acute diameter gain after PTCA.

We selected 133 healthy age and sex-matched volunteers as control subjects.

Conventional Coronary Risk Factors

The prevalence of conventional coronary risk factors such as hypertension, hyperlipidemia, diabetes mellitus, smoking and obesity were studied in all patients. Hypertension was diagnosed as systolic blood pressure of more than 160 mmHg or diastolic blood pressure of 95 mmHg, or if the patients had antihypertensive medication. NIDDM was diagnosed as the fasting blood glucose level of more than 120 mg/dl or the 2-hour blood glucose level of more than 200 mg/dl after the glucose tolerance test or if anti-diabetic medication has been prescribed for the history of NIDDM. Hyperlipidemia was diagnosed as serum cholesterol level of more than 220 mg/dl or serum triglyceride level of more than 150 mg/dl, or if the patients had antihyperlipidemial medication. Obesity was diagnosed as body-mass index exceeding 26.4 kg/m^2.

Determination of Angiotensin II Type I Receptor Genotype and Angiotensinogen

To determine the A1166C polymorphism of AT1R and T174M polymorphism of the ATN gene, genomic DNA was prepared from peripheral whole blood collected in EDTA with a DNA extraction Kit (Wako, Osaka, Japan). The primers were designed as follows : For A1166C gene polymorphism, 5'-ATAATGTAAGCTCATCCACC-3' (sense), 5'-GAGATTGCATTTCTGT-CAGT-5' (antisense) and for T174M, 5'-GATGCGCACAAGGTCCTG-3' (sense), 5'-CAGGGT-GCTGTCCACACTGGCTCGC-3' (antisense). Polymerase chain reaction (PCR) was performed as previously described[10,11]. After an initial denaturation step (1 minute at 94℃), 35 cycles of PCR consisting of 94℃ for 1 minute, 62℃ for 1 minute, and 72℃ for 2 minutes were carried out for AT1R, or 30 cycles of PCR consisting of 94℃C for 1 minute, 61℃ for 1 minute, and 72 ℃ for 1 minute for ATN. PCR products were digested with restriction enzymes (Dde I for AT1R genotype and Noc I for ATN genotype) at 37℃ overnight. The digestion products were electrophoresed on 2.0 % agarose gels and visualized by ethidium bromide staining. Patients were classified into one of three genotypes, AA, AC and CC for AT1R, TT, TM and MM for ATN as previously described[10,12]

Statistical Analysis

The χ^2 test was used to compare the distribution of genotype in CAD patients and control subjects, to compare the distribution of gene polymorphism for the severity of coronary arteriosclerosis and for the restenosis after coronary angioplasty. A level of $p<0.05$ was considered to be statistically significant.

RESULTS

Clinical Characteristics of Patients with CAD

The CAD group consisted of 93 males and 46 females with a mean age of 62 years. There were no differences in age, gender distribution, coronary risk factors, or other clinical characteristics between patients with single-vessel disease (n=52) and these with multi-vessel disease (n=78). The 107 CAD patients who received successful PTCA were divided into two groups :

Table 1. Distribution of gene polymorphism in coronary artery disease

Genotype		CAD (n=139)	C (n=133)
AT1R	AA	85	83
	AC	13	15
	CC	2	2
ATN	TT	76	79
	TM	22	20
	MM	2	1 (%)

Abbreviations: CAD: coronary heart disease patients, C : control subjects, AT1R : angiotensin II type 1 receptor, ATN : angiotensinogen.

Table 2. Relationship between genotype and the severity of coronary arteriosclerosis

Genotype		SVD (n=52)	MVD (n=78)
AT1R	AA	83	87
	AC+CC	17	13
ATN	TT	75	78
	TM+MM	25	22 (%)

SVD : single-vessel disease, MVD : multi-vessel disease, C : control subjects, AT1R : angiotensin II type 1 receptor, ATN : angiotensinogen.

Table 3. Conventional risk factors in patients with coronary artery disease treated by coronary angioplasty

Risk factor	R	NR	
Hypertension	44	36	ns
NIDDM	21	23	ns
Hyperlipidema	38	47	ns
Smoking	65	54	ns
Obesity	19	25 (%)	ns

R: patients with restenosis, NR: patients without restenosis, NIDDM: non-insulin-dependent diabetes mellitus, ns: not significant.

Table 4. Relationship between genotype and restenosis after coronary angioplasty

Genotype		R	NR	
AT1R	AA	77	91	$p<0.05$
	AC+CC	23	9	($\chi^2=3.912$)
ATN	TT	83	65	$p<0.05$
	TM+MM	17	35 (%)	($\chi^2=4.111$)

R: patients with restenosis, NR: patients without restenosis, AT1R: angiotensin II type 1 receptor, ATN: angiotensinogen.

restenosis group (n=52) and non-restenosis group (n=55). There were no differences in age or gender distribution between these two groups.

Distribution of Gene Polymorphism in Patients with CAD

Patients with the AA genotype accounted for 85 %, AC for 13 %, and CC for 2 % of those with CAD. TT genotype accounted for 76 %, TM for 22 %, MM for 2 % of those with ATN. The distributions of AT1R and ATN genotypes in CAD patients did not differ from those in control subjects (Table 1).

Relationship between Genotype and the Severity of Coronary Arteriosclerosis

As to the AT1R genotype, patients with the AA genotype accounted for 83 % of those with SVD, while AC and CC accounted for 17 %. As to the ATN genotype, the TT genotype accounted for 75 %, TM and MM accounted for 25 %. Among patients with MVD, AA genotype accounted for 87 %, AC and CC for 13 % in AT1R, while the TT genotype accounted for 78 %, TM and MM for 22 % in ATN. The distribution of AT1R and ATN genotypes in patients with SVD were similar to those in patients with MVD. Neither AT1R nor ATN genotype showed correlation with the severity of coronary arterosclerosis (Table 2).

Relationship between Genotype and Restenosis after Coronary Angioplasty

There were no significant differences in prevalence of conventional risk factors between patients with and without restenosis (Tabie 3). The AC and CC genotypes of AT1R were significantly more frequent in patients with than in those without restenosis ($p<0.05$). The TT genotype of ATN was also significantly more frequent in patients with than in those without restenosis ($p<0.05$) (Table 4).

DISCUSSION

The AT1R gene is located on human chromosome 3 q 22, and five polymorphisms have already been identified in this gene[13]. Among these polymorphisms, the polymorphism with adenine instead of cytosine at position 1166 (A1166C) has been reported to show a stronger correlation with the risk of high blood pressure than the other polymorphisms[13]. And this polymorphism was also reported to be a risk factor for myocardial infarction in 1994. The

ATN gene is located on human chromosome 1q42-43, and 15 DNA polymorphisms have been identified in this gene. Two of these mutations in exon 2, mmtation of threonine instead of methionine at position 235 (M235T) and methionine instead of threonine at 174 (T174M), are related to hypertension[14]. Furthermore, M235T polymorphism of the ATN gene was reported to be associated with increased risk of CAD[11] in 1995. In this study, we examined A1166C polymorphism of the AT1R gene and T174M polymorphism of the ATN gene. Our results suggested that the distributions of AT1R and ATN genotypes do not differ between CAD patients and control subjects. Neither AT1R nor ATN genotype was correlated with the severity of coronary arteriosclerosis. So we speculate that environmental factors have a greater contribution to the development of CAD than these genetic factors.

On the other hand, this study suggested that both AT1R and ATN gene polymorphisms may participate in the process of restenosis after coronary angioplasty. Generally percutaneous transluminal coronary angioplasty (PTCA) is currently the most effective strategy for treatment of CAD, but this interventional therapy is often accompanied by unsatisfactory events such as restenosis after PTCA. In restenosis lesion, the most common pathological finding is smooth muscle cell proliferation, but the precise mechanisms of this process have not been clarified. However, some previous studies supported the suggestion that the RAS played an important role in the process of restenosis after coronary angioplasty. Daeman et al. reported that angiotensin II induced smooth muscle cell proliferation in the balloon-injured rat arterial wall[8]. Furthermore, Rakugi et al[15]. also demonstrated the induction of vascular ACE gene expression in a rat vascular injury model, and angiotensinogen gene expression was enhanced after balloon injury of vessels relative to controls[9]. Moreover, it was reported that administration of angiotensin-converting enzyme inhibitors to normotensive rats inhibited neointima formation in balloon-injured arteries[16]. So genetic factors involved in the renin-angiotensin system are considered to contribute to restenosis after PTCA in addition to other major factors such as hypertension, hyperlipidemia and diabetes mellitus. Ohishi et al[17]. reported that the DD genotype of ACE polymorphism was a potent risk factor for restenosis after emergency angioplasty. However, some studies suggested that there are no correlations between ACE and restenosis after PTCA[18]. At the present time, it is a fact that there is a diversity of views on this question. If the genetic factors such as polymorphism gene related to the RAS are associated with raised plasma and cellular concentrations of ACE and angiotensin II, it is reasonable that polymorphism of RAS influences arteriosclerosis involving restenosis after PTCA. Because our study suggested that both AT1R and ATN gene polymorphisms may participate in the process of restenosis after coronary angioplasty, we consider that A1166C polymorphism of AT1R and T174M polymorphism of the ATN gene may be associated with the concentrations of ACE and angiotensin II in the coronary vascular wall. Moreover, we speculate that A1166C polymorphism of the AT1R gene and T174M polymorphism of the ATN gene induce smooth muscle cell proliferation in the balloon-injured arterial wall via overexpression of angiotensin II and raised angiotensin II concentration in tissue.

In this study, the number of subjects investigated was relatively small for evaluation of the relationship between genomic polymorphism and CAD. As there were very few patients with CC genotype of AT1R and MM genotype of ATN, we categorized AC and CC, TM and MM as single groups. To conclusively demonstrate that RAS related genetic factors are risk factors

for CAD, it will be necessary to study a larger number of subjects. Furthermore, we could not confirm whether the AT1R and ATN genotypes influence the concentrations of ACE and angiotensin II in patients in this study. So, further studies are necessary to elucidate the biological effects of RAS polymorphism on restenosis after PTCA. If we can demonstrate that there is indeed a relationship between RAS polymorphisms and restenosis after PTCA, drugs such as ACE inhibitors and AT1R antagonists may be useful to prevent the development of restenosis after coronary angioplasty. Our results suggest that both AT1R and ATN gene polymorphism participate in the process of restenosis after coronary angioplasty, but we could not conclusively prove the relationship between RAS polymorphisms and the development of CAD.

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