Original Article

An Interaction between Systolic Blood Pressure and Angiotensin-Converting Enzyme Gene Polymorphism on Carotid Atherosclerosis

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The insertion/deletion (I/D) polymorphism of the human angiotensin-converting enzyme (ACE) gene is a major determinant of circulating ACE activity, with the D allele being associated with higher ACE levels than the I allele. Thus, chronic exposure to high levels of circulating and tissue ACE may well predispose to vascular wall thickening and atherosclerosis. However, the effect of the ACE gene on carotid atherosclerosis remains controversial. We investigated the association between ACE gene I/D polymorphism and risk factor-dependent augmentation of carotid arterial remodeling in subjects with several risk factors for atherosclerosis. We evaluated sclerotic lesions of the common carotid artery with intima-media thickness (IMT) by ultrasonography in 184 patients (mean age $\pm$ SD, 67 ± 14 years old) and studied whether any risk factor-gene interactions were associated with carotid atherosclerosis. Out of the 184 subjects, 71 had the ACE II genotype, 87 the ID genotype and 26 the DD genotype. There was no significant difference in IMT among the three ACE genotypes. In total subjects, multiple regression analysis showed that age, total-cholesterol (T-C), and HDL-cholesterol (HDL-C) were significantly associated with IMT. However, the association between risk factors and IMT was genotype-specific. Systolic blood pressure (SBP) and HDL-C were significantly associated with IMT in ACE D carriers (DD + ID), but not in subjects with the ACE II genotype. Similarly, T-C was significantly associated with IMT only in subjects with the ACE II genotype. A general linear model of the interaction between the ACE genotype and the conventional risk factors showed that the SBP-ACE genotype interaction were significantly associated with IMT ($F=7.915$; $p=0.005$). This finding further supports the idea that analysis of risk factor-gene interaction could be a useful tool for deriving specific predictive information about the development of atherosclerosis. (*Hypertens Res* 2002; 25: 875–880)

Key Words: risk factor, gene, interaction, ACE, polymorphism

Introduction

The insertion/deletion (I/D) polymorphism of the human angiotensin-converting enzyme (ACE) gene is a major determinant of circulating ACE activity, with the D allele being associated with higher ACE levels than the I allele (I). ACE catalyzes the conversion of angiotensin I to angiotensin II and the breakdown of bradykinin to kinin degradation prod-

ucts (2). The two peptide hormones have opposite effects on vascular tone, smooth muscle cell proliferation, and extracellular matrix production (3–5). Thus, chronic exposure to high levels of circulating and tissue ACE may well predispose to vascular wall thickening and atherosclerosis.

The D allele has been suggested to be a potent risk factor for myocardial infarction (6), coronary heart disease (7), coronary artery restenosis (8) and left ventricular hypertrophy (9), although conflicting results have also been reported

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(10). Several recent studies have further investigated the possible influence of I/D polymorphism of the ACE gene on carotid atherosclerosis. The D allele of the ACE gene was found to be a risk factor for carotid intima-media thickness (IMT) (11–14), although the ACE I/D polymorphism was reported not to be a risk predictor of carotid wall thickening (15–18). Therefore, the effect of the ACE gene on carotid atherosclerosis remains controversial.

One of the possible reasons for the lack of replicable results is that most of these studies have ignored the genetic and environmental context of the studied populations (19). We recently observed that risk factor-gene interaction can influence carotid atherosclerosis (20). The conflicting evidence regarding an association between the ACE gene and atherosclerosis may suggest an interaction between this gene and the risk factors for atherosclerosis. In the present study, we investigated the association between ACE polymorphism and risk factor-dependent augmentation of carotid arterial remodeling in subjects with several risk factors for atherosclerosis.

Subjects and Methods

Subjects

Participants were consecutively enrolled from among in-patients in the medical department of Nomura Municipal Hospital between August 1999 and December 2000. All participants had undergone carotid ultrasound examination for evaluation of possible atherosclerosis. The causes of hospitalization were respiratory disorders including pneumonia in 43, diabetes mellitus in 26, digestive disorders in 25, neurological disorders other than stroke in 23, infections in 23, neoplasm in 14, hypertension in 7, collagen or skin disorders in 5, metabolic disorders in 4, and miscellaneous diseases in 14 cases. All subjects were free of any cardiac or renal disorders. Those who had a history of transient ischemic attack, cerebral infarction, angina pectoris, or myocardial infarction were excluded from the study. None of the participants had ever taken any anti-hyperuricemic drugs or anti-hypertensive drugs. However, six patients had been treated with an anti-hyperlipidemic drug and all diabetic patients ($n = 35$) had been treated with either sulfonylurea or insulin in addition to diet modification.

Informed consent for the procedures herein was obtained from each patient. All procedures were approved by the Ethics Committee of Ehime University School of Medicine.

Ultrasound Image Analysis

An ultrasonograph (EUB-565, Hitachi, Tokyo, Japan) equipped with a 7.5 MHz linear type B-mode probe was used by a specialist in ultrasonography to evaluate sclerotic lesions of the common carotid arteries on a day as close as possible to—and within 3 days after—the day of the blood

biochemistry analyses. Patients were asked to assume the supine position, and the bilateral carotid arteries were observed obliquely from the front and rear directions. We measured IMT on the far wall of the bilateral common carotid artery at about 10 mm proximal to the bifurcation on the B-mode monitor, and used the mean values for study.

Other Various Risk Factors

We measured blood pressure in the right upper arm of patients in a sedentary posture using a standard sphygmomanometer. Cigarette smoking was quantified based on daily consumption and duration of smoking. In the blood biochemistry analyses, total-cholesterol (T-C), triglyceride (TG), HDL-cholesterol (HDL-C), uric acid and blood sugar were measured upon fasting within 2 days after admission.

ACE Genotype Analysis

The I/D polymorphism of the ACE gene was detected by polymerase chain reaction (PCR) according to the method of Rigat *et al.* (21) using the primers 5'-CTGGAGACCACTCC CATCCTTTCT-3' and 5'-GATGTGGCCATCACATTCGT CAGAT-3'. Misclassification of ID to DD was checked by performing a second independent PCR amplification of DD samples using an insertion-specific primer (22).

Statistical Analysis

All values are expressed as the mean $\pm$ SD except where specified. Statistical analysis was performed using SPSS 10.0J software (Statistical Package for Social Science, Inc., Chicago, USA). The prevalence among genotypes was compared by the χ^2 test. The differences among groups were analyzed by the Mann-Whitney *U*-test. Analysis of covariance was employed to evaluate the difference of risk factor-genotype interactions in IMT. As risk factors, we evaluated sex, age, body mass index (BMI), smoking, systolic blood pressure (SBP), diastolic blood pressure (DBP), T-C, TG, HDL-C, uric acid and diabetes mellitus. A general linear model was employed to evaluate the significant contribution of risk factor-gene interactions to IMT. Values of $p < 0.05$ were considered to indicate statistical significance.

Results

Background of Subjects with the Three ACE Genotypes

The breakdown of the 184 subjects (mean age, 67 ± 14 years) by gender was 86 males (65 ± 15 years) and 98 females (68 ± 14 years). Table 1 shows their background factors classified by ACE gene polymorphism. Seventy-one subjects had the ACE II genotype, 87 the ID genotype and 26 the DD genotype. The distribution of ACE genotypes was consistent with that of a published report in Japanese sub-

Table 1. Characteristics of the Subjects by ACE Genotype

Risk factor	II genotype	ID + DD genotype	Total	<i>p</i> value
<i>n</i>	71	113	184	
Male (<i>n</i> (%))	35 (49.3)	51 (45.1)	86 (46.7)	0.650
Age (years)	69 ± 13	65 ± 15	67 ± 14	0.070
Body mass index (kg/m ²)	21.4 ± 4.5	21.7 ± 3.3	22 ± 3.8	0.277
Smoking (pack-years)	14 ± 19	14 ± 22	14 ± 21	0.761
Systolic blood pressure (mmHg)	128 ± 20	127 ± 19	127 ± 19	0.540
Diastolic blood pressure (mmHg)	74 ± 12	76 ± 10	75 ± 11	0.355
Total-cholesterol (mg/dl)	175 ± 40	177 ± 42	176 ± 41	0.966
Triglyceride (mg/dl)	92 ± 59	87 ± 55	89 ± 57	0.723
HDL-cholesterol (mg/dl)	51 ± 20	54 ± 17	53 ± 18	0.114
Uric acid (mg/dl)	5.0 ± 1.9	4.6 ± 1.8	4.8 ± 1.9	0.278
Diabetes mellitus (<i>n</i> (%))	16 (22.5)	19 (16.8)	35 (19.0)	0.342
Carotid intima-media thickness (× 10 ⁻¹ mm)	9.2 ± 1.6	9.1 ± 1.9	9.1 ± 1.8	0.816

Values are mean ± SD. HDL, high-density lipoprotein. Smoking (pack-years) was expressed as the number of cigarettes per day × the number of smoking years. The Mann-Whitney *U*-test was used to determine the significance of differences between the ACE II genotype and the ID + DD genotypes.

Table 2. Multivariate Linear Regression Analysis for Intima-Media Thickness with Conventional Risk Factors and the ACE Genotype

Risk factor	II genotype	ID + DD genotypes	Total
<i>n</i>	71	113	184
Male (%)	0.003 (0.982)	0.053 (0.605)	0.033 (0.680)
Age (years)	0.447 (0.001)	0.552 (< 0.001)	0.515 (< 0.001)
Body mass index (kg/m ²)	- 0.160 (0.360)	0.068 (0.374)	- 0.028 (0.687)
Smoking	0.110 (0.417)	0.122 (0.183)	0.131 (0.077)
Systolic blood pressure (mmHg)	- 0.079 (0.674)	0.228 (0.008)	0.129 (0.106)
Diastolic blood pressure (mmHg)	0.020 (0.901)	- 0.017 (0.842)	- 0.023 (0.758)
Total-cholesterol (mg/dl)	0.300 (0.037)	0.091 (0.319)	0.167 (0.030)
Triglyceride (mg/dl)	0.164 (0.286)	- 0.003 (0.975)	0.016 (0.824)
HDL-cholesterol (mg/dl)	- 0.157 (0.238)	- 0.326 (< 0.001)	- 0.255 (< 0.001)
Uric acid (mg/dl)	0.198 (0.099)	0.063 (0.437)	0.125 (0.061)
Diabetes mellitus	0.100 (0.394)	0.100 (0.183)	0.061 (0.321)
ACE genotype	—	0.110 (0.072)	
<i>r</i> ²	0.315 (0.013)	0.517 (< 0.001)	0.406 (< 0.001)
	<i>F</i> = 2.471	<i>F</i> = 9.824	<i>F</i> = 9.732
	(df 11, 59)	(df 11, 101)	(df 12, 171)

Values are the standard regression coefficients (*p* values). HDL, high-density lipoprotein; *r*², multiple coefficient of determination. A dominant model of the D allele (II = 0, ID + DD = 1) was used for the ACE genotype.

jects (23) and was in agreement with Hardy-Weinberg equilibrium (*p* = 0.941). Among the three genotypes of ACE, there were no differences in background factors including sex, age, BMI, smoking, SBP, DBP, lipid levels, uric acid or the presence of diabetes mellitus.

ACE Genotypes and Conventional Risk Factors for IMT

To find possible gene and risk factor interactions for IMT, multiple regression analysis for IMT was performed with

risk factors in subjects with a specific genotype of the ACE gene polymorphism (Table 2). Since all diabetic patients had been treated, category for diabetes was used for the analysis. The results showed that age and T-C were significantly associated with IMT in subjects with the ACE II genotype, and that age, SBP and HDL-C were significantly associated with IMT in ACE D carriers (ID + DD). SBP, T-C and HDL-C themselves were not significantly different between subjects with the ACE II genotype and ACE D carriers (Table 1). Analysis of covariance adjusted for other risk factors showed

Fig. 1. Genotype-dependent linear regression of the effects of carotid intima-media thickness on systolic blood pressure. Analysis of covariance showed that the two regression lines were significantly different ($F[1,174] = 4.082$; $p = 0.045$). (a) ACE II genotype, $n = 71$, $r = 0.085$, $p = 0.085$. (b) ACE ID + DD genotypes, $n = 113$, $r = 0.254$, $p = 0.007$.

that the regression line between SBP and IMT was significantly different between subjects with the ACE II genotype and ACE D carriers (SBP - ACE genotype $F[1,174] = 4.082$; $p = 0.045$) (Fig. 1). On the other hand, analysis of covariance failed to demonstrate significant differences between the two regression lines in T-C and carotid IMT ($F[1,174] = 2.119$; $p = 0.147$) as well as between those in HDL-C and carotid IMT ($F[1,174] = 3.736$; $p = 0.055$).

Table 3. General Linear Model for Carotid Intima-Media Thickness with Interactions between the ACE Genotype and Risk Factors

Risk factor	<i>F</i>	<i>p</i> value
Age (years)	75.43	< 0.001
Smoking (pack-years)	6.334	0.013
Systolic blood pressure (mmHg)	1.557	0.214
Total-cholesterol (mg/dl)	9.403	0.003
HDL-cholesterol (mg/dl)	17.21	< 0.001
Uric acid (mg/dl)	5.410	0.021
Diabetes mellitus	2.868	0.092
ACE genotype	0.592	0.443
Interaction of ACE genotype to:		
Systolic blood pressure (mmHg)	7.915	0.005
Total-cholesterol (mg/dl)	1.773	0.185
HDL-cholesterol (mg/dl)	3.555	0.061

A dominant model ($II = 0$, $ID + DD = 1$) was used for the ACE genotype.

Risk Factor-ACE Gene Interaction for IMT

To further investigate whether the interaction between ACE genotype and the conventional risk factors could influence IMT, a general linear model for IMT was analyzed using the following parameters: age, smoking, SBP, T-C, HDL-C, uric acid, presence of diabetes mellitus, and ACE gene polymorphism, including interactions between the ACE gene and SBP, T-C, and HDL-C (Table 3). Since the correlation coefficients with carotid IMT were not statistically significant for sex, body mass index, diastolic blood pressure or triglycerides, these factors were not included in the general linear model. The results revealed that the interaction between SBP and ACE genotype ($F = 7.915$, $p = 0.005$) was significantly associated with IMT, in addition to age, smoking, T-C, HDL-C and uric acid. This finding indicates that the association between SBP and IMT was significantly different between subjects with the ACE II genotype and D carriers.

Discussion

This study examined the relation between ACE gene polymorphism and carotid atherosclerosis evaluated by ultrasonography in patients with risk factors for IMT. We revealed that the presence of a D allele itself was not a significant risk factor for IMT thickening after adjustment for conventional risk factors including sex, age, SBP, DBP, T-C, HDL-C, uric acid and presence of diabetes mellitus. However, the ACE genotype was found to interact with SBP to influence IMT.

Early studies reported an association between ACE I/D polymorphism and carotid atherosclerosis in the general Caucasian population (11, 14) as well as in Japanese patients with diabetes (13). However, recent studies have failed to

demonstrate such an association in the general population (15–18, 24–26). In the present study, we also found no difference in carotid IMT among ACE genotypes, suggesting that gene polymorphism itself was not a sufficiently potent parameter to affect carotid atherosclerosis. The classical risk factors for development of atherosclerosis are aging, obesity, smoking, hypertension, hyperlipidemia, and diabetes mellitus. Since atherosclerosis is a multifactorial disorder including genetic predisposition in addition to these risk factors, gene-risk factor interaction could, in various ways, decrease or enhance the absolute risk in individual subjects. These interactions may account for the contradictory results of previous studies of ACE polymorphism and carotid atherosclerosis (15). In the present study, we found that the regression of IMT on SBP was significantly different among ACE genotypes. The slope of the linear regression on SBP was steeper in subjects who were D carriers than in subjects with the ACE II genotype, suggesting that the effect of an increase of SBP on IMT thickening is more pronounced in D carriers. This finding could not have been obtained by a simple comparison among genotypes.

IMT thickening of the carotid artery has been reported to be associated with increased plasma ACE activity (27). In Japanese patients with non-insulin-dependent diabetes, ACE activity is significantly higher in subjects with ACE D carriers than in those with the ACE II genotype (13). It has also been demonstrated that ACE gene polymorphism accounts for half of the variance of the ACE serum activity (1). Recent reports showed that local ACE activity in tissues such as the ventricles of organ donors without cardiac disease (28) and blood vessels (29) was higher in ACE D carriers. This elevation may lead to an increased risk of atherosclerosis by modulating the opposing effects of angiotensin II and kinins on endothelial function, smooth muscle cell proliferation, extracellular matrix deposition, and vascular reactivity in the vessel wall (3–5). A genetically determined chronic exposure to elevated ACE activity might be involved in structural changes of the arterial wall and vascular wall thickening, especially in the presence of risk factors such as hypertension. In large population-based studies performed both in Framingham (30) and Suita (31), it has been demonstrated that the ACE DD genotype is associated with an independent risk of hypertension in male subjects. In the present study, we did not observe any sex-specificity in the interaction between the ACE gene and SBP (data not shown). However, this finding may have been related to the fact that many of our female patients were post-menopausal. Further studies with a larger number of subjects will be needed to evaluate this possibility.

Recently, we also observed a genotype-specific association between adhesion molecules and carotid atherosclerosis (32). Taken together, these findings indicate that ACE gene polymorphism may directly or indirectly influence the progression of carotid atherosclerosis.

Hypertension is one of the potent risk factors for cardio-

vascular diseases (33). In the present study, there was no significant difference in the levels of blood pressure between subjects with the ACE II genotype and D carriers. However, the effect of SBP on carotid IMT was significantly different between the two groups. These findings may indicate that high blood pressure is a genotype-specific risk factor for cardiovascular diseases. Prospective observation in a larger population will also be needed to confirm this hypothesis.

There were several important limitations to the present study. Since all participants were hospitalized patients, we cannot rule out the possibility that underlying diseases affected the results. Although participants were free from anti-hypertensive drugs, all diabetic patients and six patients with hyperlipidemia had been treated with drugs. We cannot eliminate the possible effect of these medications on the present findings. These points need to be addressed again in a large population-based sample in a prospective fashion.

In summary, our results suggest that ACE gene polymorphism itself was not potent enough to lead to the development of carotid atherosclerosis. However, the interaction with SBP, one of the most potent risk factors of atherosclerosis, could promote the effect of the gene, thereby resulting in carotid atherosclerosis. This finding further supports the idea that risk factor-gene interaction could allow us to isolate the specific mechanism underlying the genetic factors involved in the development of atherosclerosis.

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