Significance of the Deletion Polymorphism of the Angiotensin Converting Enzyme Gene as a Risk Factor for Myocardial Infarction in Japanese

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An association study of the insertion/deletion (*I/D*) polymorphism of the angiotensin converting enzyme (ACE) gene with myocardial infarction (MI) was performed in middle-aged Japanese men. The *DD* genotype was significantly more frequent in 101 MI subjects (0.42) than in 102 matched control subjects (0.16) compared with the two other genotypes (*ID* and *II*) ($p < 0.00005$). A significant association was observed not only in the low-risk group but also in the high-risk group who were defined according to plasma total cholesterol ≥ 220 mg/dl and body mass index ≥ 26 kg/m². These results indicate that ACE/*DD* genotype is a potent and important risk factor for MI in Japanese. (*Hypertens Res* 1994; 17: 55-57)

Key Words: myocardial infarction, angiotensin I-converting enzyme, genotype, insertion/deletion polymorphism

Coronary heart disease (CHD) remains a major public health problem in industrialized countries because of its contribution to total mortality. Large international differences in the trends in CHD incidence and mortality have been reported (1). A decline has occurred in the USA, and an increase has been noted in Eastern Europe (2). In Japan, CHD-related mortality has been decreasing, but the incidence of CHD appears to be gradually increasing. A number of risk factors for coronary atherosclerosis have been well documented based on their relationship to the incidence of CHD in epidemiologic studies. Included among the major risk factors are hyperlipidemia, obesity, hypertension, cigarette smoking, male sex, and diabetes mellitus (3). Recently, one genotype of a polymorphism in the gene for angiotensin converting enzyme (ACE) has been reported to be a new potent risk factor for myocardial infarction (MI) (4).

The ACE gene has been considered as a candidate gene which may contribute to the development of hypertension and cardiovascular diseases. The 287 bp insertion/deletion (*I/D*) polymorphism located in intron 16 of the ACE gene has been reported to be associated with marked differences in serum ACE levels in unrelated healthy individuals (5). This apparent association has been noted only in severely hypertensive Caucasians (6), but not in Caucasian offspring in their 20s with the highest

blood pressure for their age group and two hypertensive parents (7,8), nor in Japanese hypertensives (9). Recently, Cambien *et al.* reported that the *DD* genotype, which is associated with higher levels of circulating ACE than the *ID* and *II* genotypes, is significantly more frequent in patients with MI than in controls, especially among subjects normally considered to be at low risk (4).

The present report describes an association study of the *I/D* polymorphism in the gene for ACE with MI in middle-aged Japanese men.

Methods

Patient selection: The study population consisted of 203 unrelated male Japanese subjects, 65 years of age or younger, from Osaka University Hospital, Sakurabashi-Watanabe Hospital, and Sakuragaoka Hospital. One-hundred and one of the subjects were patients who had suffered at least one MI during the period from January 1990 through March 1993. All of the patients were diagnosed as having MI by coronary angiography, electrocardiogram criteria, and measurements of heart-specific serum enzymes. One-hundred and two of the matching control subjects were normal volunteers and outpatients at the same 3 hospitals. None had a history of CHD, and all tested normal on electrocardiograms both during rest and physical stress. The mean age

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of the patients was 53.6 years (SD 6.8), and that of the controls was 53.5 years (SD 9.2). Informed consent was obtained from the subjects.

Detection of the *I/D* polymorphism of the ACE gene: DNA was extracted from peripheral blood leukocytes as described previously (10). DNA concentrations were measured by absorbance at 260 nm. Polymerase chain reaction (PCR) was performed according to the method of Rigat *et al.* (11). The sequence of the sense primer and the antisense primer were 5'-CTGGAGACCACTCCCATCCTT-TCT-3' and 5'-GATGTGGCCATCACATTCGTC-AGAT-3', respectively. PCR was performed in a final volume of 10 μ l which contained 20 ng of genomic DNA, 4 pmol of each primer, 200 μ M each of the four dNTP, 1.5 mM MgCl₂, 20 mM KCl, 500 μ g/ml bovine serum albumin, 50 mM Tris-HCl, pH 8.5, and 0.3 units of Taq polymerase (Perkin Elmer Cetus, Norwalk, CT, USA). Amplification was carried out in a Gene Amp PCR System 9600 (Perkin Elmer Cetus, Norwalk, CT, USA) for 30 cycles with steps of denaturation at 94°C for 60s, annealing at 55°C for 30s, and extension at 72°C for 90s. The PCR products were electrophoresed in 2% agarose gels, and DNA was visualized directly with ethidium bromide staining.

Statistical analysis: Values are expressed as mean $\pm$ SD. Allele frequencies were deduced from genotype frequencies, and the difference between groups was tested by χ^2 analysis with 1 degree of freedom. The significance of the difference in variables of risk factors between the *I/D* genotypes of the ACE gene was assessed by Student's *t*-test. A value of $p < 0.05$ was considered statistically significant.

Results

PCR allowed the detection of the 287 bp *I/D* polymorphism of the ACE gene that was evident as a

490 bp fragment (insertion: allele *I*) and a 190 bp fragment (deletion: allele *D*). Frequencies for *DD*, *ID*, and *II* genotypes were 42, 33 and 26 in the MI subjects, and 16, 44 and 42 in the control subjects, respectively ($\chi^2 = 17.8$, $p < 0.0002$) (Table 1). The *DD* genotype was significantly more frequent in the MI group than in the control group compared with the two other genotypes (*ID* and *II*) ($p < 0.00005$). The odds ratio (an estimate of the relative risk of MI between subjects with the *DD* genotype and subjects with the *ID* or *II* genotypes) was 3.8.

All subjects were divided into two groups according to risk status. The low-risk group included subjects with a plasma total cholesterol level lower than 220 mg/dl, those not taking hypolipidaemic drugs, and those with a body mass index < 26 kg/m². In this group, the association between the *DD* genotype and MI was highly significant ($p < 0.0008$), and the odds ratio was 4.3. Further, even in the high-risk group, which included all subjects who were not in the low-risk group, this association was also statistically significant ($p < 0.02$), and the odds ratio was 3.4 (Table 1).

Several variables, which are considered to be risk factors for MI, were compared between the *I/D* genotypes of the ACE gene in both the MI and control groups (Table 2). Plasma HDL-cholesterol levels were significantly higher in the control group compared with those in the MI group in *ID* and *II* genotypes. However, no difference could be detected between genotypes in either group. For plasma total cholesterol level, body mass index, and systolic and diastolic blood pressure, there was no difference between genotypes.

Discussion

The age-adjusted death rates from CHD in Japan are about one-fifth of those in the USA (1). However, recently, with the adaptation of the cul-

Table 1. Frequencies of ACE genotypes in MI and control subjects

	Total subjects		Low-risk group*		High-risk group**	
	MI	Controls	MI	Controls	MI	Controls
Genotypes						
<i>DD</i>	42	16	26	10	16	6
<i>ID</i>	33	44	19	19	14	25
<i>II</i>	26	42	9	27	17	15
	$\chi^2 = 17.8 (p < 0.0002)$		$\chi^2 = 16.6 (p < 0.0003)$		$\chi^2 = 7.1 (p < 0.03)$	
<i>DD</i>	42	16	26	10	16	6
<i>ID + II</i>	59	86	28	46	31	40
	$(p < 0.00005)$		$(p < 0.0008)$		$(p < 0.02)$	
Odds ratio [†]	3.8		4.3		3.4	
Alleles						
<i>D</i>	0.58	0.37	0.66	0.35	0.49	0.39
<i>I</i>	0.42	0.63	0.34	0.65	0.51	0.61
	$(p < 0.00004)$		$(p < 0.000005)$		$(p < 0.3)$	

*Subjects with BMI < 26 kg/m², total cholesterol level < 220 mg/dl, and not taking hypolipidemic drugs. **Subjects not in the low-risk group. [†] The odds ratio is an estimate of the relative risk of MI between subjects with the *DD* genotype and subjects with the *ID* or *II* genotype.

Table 2. Comparison of several variables between ACE/ID genotypes in MI and control subjects

ACE genotype	MI subjects			Control subjects		
	DD	ID	II	DD	ID	II
Number of subjects	42	33	26	16	44	42
Age (y. o.)	53.8±5.9	53.2±6.9	53.8±7.7	51.0±10.4	54.4±8.4	53.5±9.5
Total cholesterol (mg/dl)	209±26	204±24	213±30	182±50	207±35	197±40
HDL cholesterol (mg/dl)	39±11	38±10	37±9	43±18	50±11*	49±11*
Body mass index (kg/m ²)	24.1±2.7	24.1±3.0	24.8±2.8	23.8±3.7	24.1±3.0	23.4±3.3
Systolic blood pressure (mmHg)	134±21	138±21	133±17	139±19	134±16	132±18
Diastolic blood pressure (mmHg)	81±14	83±15	79±13	86±12	85±9	84±11

Values are expressed as mean ± SD. * $p < 0.001$ vs. MI subjects.

All comparisons are by unpaired *t*-test.

ture of western industrialized countries and the rapid increase in the elderly population, the prevalence of CHD has been gradually increasing, and the management of CHD in view of its risk factors has become one of the important issues for its prevention. Cambien *et al.* reported that the ACE/DD genotype is a new potent risk factor for MI in middle-aged Caucasian men, particularly in subjects normally considered to be at low-risk, and hypothesized that the mechanism by which the ACE polymorphism affects the risk of MI is by modulating the level of angiotensin II and bradykinin in the coronary arteries (4). The present study demonstrated that ACE/DD genotype is significantly more frequent in patients with MI than in controls in middle-aged Japanese men, which confirms the results of Cambien *et al.* that this ACE/DD genotype is a new potent risk factor for MI in Japanese, also.

The most interesting finding in this study is that the DD genotype was significantly more frequent in patients with MI even in the high-risk group, in which Cambien *et al.* could not detect a significant relationship between the ACE/ID polymorphism and MI (4). This difference may be explained by the cultural differences. Among the most obvious cultural differences between Japanese and Caucasians are total calories, fat content of the diet, and the amount of physical work (12). While the Japanese life-style has become more western, the prevalence of severe hyperlipidemia and marked obesity is still relatively low in Japan compared with that in USA and Western Europe. Thus, these risk factors may not constitute such a substantial contribution to the development of coronary arteriosclerosis in Japanese compared with Caucasians. Therefore, the ACE/DD genotype is significantly related to MI even in the high-risk group. Cultural factors are generally more important than genetic determination of CHD, but this may not apply in the case of Japanese.

In conclusion, the ACE/DD genotype is a potent and important risk factor for MI in middle-aged Japanese men, not only in low-risk subjects but also in high-risk subjects.

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