Association of ACE Gene Polymorphisms with Coronary Artery Disease in a Northern Area of Japan

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SUMMARY

The insertion/deletion DNA polymorphism of the gene coding human angiotensin converting enzyme (ACE) was examined in 109 patients with coronary artery disease (CAD) and 93 non-coronary subjects (NCS) living in a northern part of Japan. The presence of risk factors including age, hypertension, hypercholesterolemia, tobacco use, diabetes mellitus and hyperuricemia were also examined. An insertion (I) / deletion (D) polymorphism of the ACE-gene was determined by the polymerase chain reaction with oligonucleotide primers encompassing the polymorphic region in intron 16. The template DNA was isolated from peripheral blood leukocytes of patients. The frequency of the D-allele in NCS was 0.27, significantly lower than that reported in Caucasians or in Japanese living in the Osaka area. The frequency of the D-allele in patients with myocardial infarction (MI) and angina pectoris was 0.39 and was higher than that in NCS. The frequencies of genotypes DD, ID, and II were 17.8, 43.3 and 38.9%, respectively, in CAD except in young patients (below 40 years of age) with MI and AP groups, and 6.5, 40.9 and 52.7%, respectively in NCS ($p < 0.05$ between CAD and NCS). Young MI showed similar frequencies in ACE gene polymorphisms to those in NCS, a pattern which differed from that seen in subjects with CAD ($p < 0.05$). The numbers of risk factors did not alter the frequency of ACE gene genotype among patients with CAD, however, in normotensives, the odds ratio of DD-genotype was significantly increased to 3.4. Accordingly, ACE gene polymorphism may be associated with morbidity from CAD in Japanese living in northern Japan as has been noted in Caucasians, despite the lower frequencies of the D-allele in the Japanese population. (Jpn Heart J **36**: 557–564, 1995)

Key words: Angiotensin I-converting enzyme Insertion/deletion polymorphism Myocardial infarction Risk factors

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This study was supported in part by grants-in-aid for Scientific Research (No. 06274202, 06454284) from the Ministry of Education, Science and Culture, Japan, grants for Research on Cardiovascular Disease from the Ministry of Health and Welfare, Japan, and a grant from Yamagata prefecture.

Received for publication May 15, 1995.

Accepted June 21, 1995.

CAMBIEN and colleagues described the rationale of DNA-typing to determine a risk profile for cardiovascular disease.[1,2] With regard to the insertion (I) / deletion (D) DNA polymorphism of the gene coding human angiotensin converting enzyme (ACE), the frequency of deletion polymorphism or the D-allele was significantly greater in patients with myocardial infarction than in controls.[1] The DD genotype is associated with higher concentrations of circulating ACE.[3,4] These findings require attention, since administration of an ACE inhibitor to patients with asymptomatic left ventricular dysfunction decreased the risk of development of heart failure and also the risk of a recurrent myocardial infarction.[5] Association of polymorphism with a parental history of fatal myocardial infarction shows the importance of the genetic variation in the ACE locus.[2] The increased risk for myocardial infarction in patients with the deletion polymorphism seems to be independent of other risk factors, particularly the usual lipid variables.[1] However, the risk profile for coronary artery disease differs with the country.[6] In China, the primary risk factors are mainly tobacco use and hypertension; in Japan, tobacco use remains the most important and in Germany, 38% of 6025 persons had 3 or more risk factors, especially obesity and hyperlipidemia.[7] As there are ethnic differences in these risk factors and in morbidity and mortality for ischemic heart disease,[8] studies on non-Caucasians have to be done. We report here an association between ACE-gene polymorphism and risk factors for coronary artery disease in Japanese living in northern Japan.

Materials and Methods

Subjects: Ninety consecutive Japanese patients (mean ± SD age, 63 ± 10 years) referred for cardiac catheterization to the First Department of Internal Medicine, Yamagata University Hospital and diagnosed as cases of myocardial infarction or angina pectoris were enrolled in a study on coronary artery disease. Sixty of the 90 patients had myocardial infarction (MI) and 30 were diagnosed as having angina pectoris (AP). Data on another 19 patients (36 ± 4 years) with myocardial

Table I. Distribution of ACE Genotypes and Frequency of D Allele in Non-coronary Subjects

		ACE-genotypes			
Groups	Age	DD	ID	II	D Allele
Subjects under 40 y	29 ± 6	1	12	11	0.29
Subjects over 56 y	64 ± 5	1	12	19	0.22
Patients	61 ± 6	4	14	19	0.28
Total		6	38	49	0.27

The group of patients includes bronchial asthma, sarcoidosis, lung cancer and chest pain syndrome. DD = deletion/deletion; ID = insertion/deletion; II = insertion/insertion.

infarction (young MI), were collected consecutively from lists of cardiac catheterization subjects in three cardiovascular centers (Yamagata University Hospital, Yamagata Prefectural Central Hospital and Ishinomaki Red Cross Hospital). In all cases, the parents of each patient were residents of the Yamagata area or the northern part of Japan. Since the age was different between MI or AP and young MI, the young MI group was analyzed separately from the coronary artery disease group (CAD) which included both MI and AP.

Non-coronary subjects included 1) residents under age forty years ($n = 24$, 29 ± 6 years), 2) residents over age 56 years ($n = 32$, 64 ± 5 years), 3) patients with no cardiac disease ($n = 37$, 61 ± 6 years), but who had bronchial asthma, lung cancer, sarcoidosis or chest pain syndrome (Table I). None had a history of coronary artery disease and electrocardiograms during rest and physical exercise were normal. Chest pain syndrome was diagnosed in cases with normal coronary angiograms both at rest and during an ergonovine study. Patients with hypertrophic or dilated cardiomyopathy were excluded from the group of non-cardiac diseases.[9,10] All subjects were the offspring of parents who were residents of the Yamagata area or of the northern part of Japan. Informed consent was obtained from the subjects.

DNA extraction and ACE gene polymorphism: DNA was prepared from leukocytes in peripheral blood using Isoquick kits (Microprobe Corporation, Bothell, WA, USA). Before initiation of the polymerase chain reaction (PCR), DNA concentrations were measured by absorbance at 260 nm and the DNAs were run on 0.4% agarose gels. These DNAs were used as the template for PCR amplification of the intron 16 of the ACE gene, using two primers designed to encompass the polymorphic region;[11] sense oligo 5′CTGGAGACCACTCCC-ATCCTTTCT3′ and anti-sense oligo: 5′GATGTGGCCATCACATTCGT-CAGAT3′. The PCR reaction contained DNA template 100 μg/m*l*, 30 pmol of each primer, 200 μmol of dNTPs, 1.25 units of AmpliTaq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT, USA), and 2 mM $MgCl_2$. DNA was amplified for 30 cycles with denaturation at 94°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 2 min, and a final extension time of 5 min using a PC-700 thermal cycler (Astec Corp., Fukuoka, Japan). PCR products were analyzed on ethidium bromide-stained agarose gel electrophoresis for allele identification, where a 190 bp fragment (D) indicates the absence of the insertion and a 490 bp fragment (I) presence of the insertion.[11]

Evaluation of coronary risk factors: Routine laboratory tests and coronary risk factors were evaluated. Hyperlipidemia was defined as a total cholesterol value over 220 mg/d*l* prior to ingestion of a meal.[12] Hypertension met the WHO criteria of over 160 mmHg in systole or 90 mmHg in diastole.[13] Diabetes mellitus was diagnosed based on the results of a 75 g oral glucose tolerance test.[14] Use of

tobacco was considered a significant risk factor at over 200 points on the Brinkman Index.[15] We used the standard for our hospital to diagnose hyperuricemia where over 8.7 mg/d*l* in men and over 5.4 mg/d*l* in women is considered to be significantly elevated.

Statistics: Numerical data are presented as means ± SD. Differences in the distribution of the DD, ID, or II genotypes between the two groups of subjects were determined by multiple group contingency table analysis. Difference in the distribution of D and I alleles between groups was tested using the chi-square test. To determine independency of ACE gene polymorphism from other coronary risk factors, we used the chi-square test. P values < 0.05 were considered statistically significant.

Results

The frequencies of the D and I alleles of the ACE gene in our non-coronary subjects were 0.27 and 0.73, respectively. The frequency of the DD genotype in the non-coronary subjects was 6%. Since we included several categories in non-coronary subjects, we separately calculated the frequencies of D/I alleles and the genotype polymorphisms. Although the number was small in each category, a lower frequency of the D-allele was clearly observed (Table I).

Table II. Distribution of ACE Genotypes and Frequency of D Allele in Patients with Coronary Artery Disease

		ACE-genotypes			
Groups	Age	DD	ID	II	D Allele
MI	64 ± 10	10	26	24	0.38
AP	61 ± 7	6	13	11	0.42
Young MI	38 ± 6	3	5	11	0.29

Myocardial infarction was diagnosed according to the MONICA category (J Clin Epidemiol **41**: 105-114, 1988). MI = myocardial infarction; AP = angina pectoris.

Table III. % Incidence of Coronary Risk Factors in Individual ACE Genotype in Patients with CAD (Except Young MI)

	DD (%)	ID (%)	II (%)	Prevalence in CAD (%)
Hypertension	31	51	71	56
Hypercholesterolemia	50	36	46	42
Smoking habit	50	56	60	57
Diabetes mellitus	25	26	20	23
Hyperuricemia	13	18	17	17

CAD = coronary artery disease.

Table IV. Number of Risk Factors in ACE Genotype in Patients with CAD (Except Young MI)

Number of risk factors	DD	ID	II	χ^2-test
~2	12	27	26	$\chi^2 = 0.31$, N.S.
≧3	4	12	9	

N.S.=not significant.

Table V. Distribution of ACE Genotypes in Patients with Hypertension, Hypercholesterolemia, and Smoking Habits in Coronary Artery Disease (Except Young MI)

	Genotypes			
	DD	ID	II	χ^2-test
Hypertension (+)	5	20	25	$\chi^2 = 7.69$, $p < 0.03$
Hypertension (−)	11	19	10	
Hypercholesterolemia (+)	8	14	16	$\chi^2 = 1.21$, N.S.
Hypercholesterolemia (−)	8	25	19	
Smoking habit (+)	9	22	21	$\chi^2 = 0.25$, N.S.
Smoking habit (−)	7	17	13	

N.S. = not significant.

The frequency of distribution of ACE genotypes and D-allele in patients with coronary artery disease is summarized in Table II. The frequencies of the D-allele and the DD genotype in patients with coronary artery disease did not differ between those with myocardial infarction and those with angina pectoris and were significantly higher than those in the non-coronary group (Table I) and younger patients with myocardial infarction (Table II). The odds ratio as an index of the relative risk of coronary artery disease (MI + AP) with the DD genotype to non-coronary subjects with the genotypes (II + ID) was 3.1.

The frequencies of risk factors with each ACE genotype are summarized in Table III. Independency of the ACE genotype from other risk factors was noted. The number of risk factors did not correlate with ACE genotypes (Table IV). As hypertension, hypercholesterolemia and tobacco use were present in 56, 42 and 57% of CAD subjects, respectively, the frequency of ACE polymorphisms was examined with the presence or absence of each risk factor (Table V). In patients (>40 years) with CAD, the DD genotype was significantly more prevalent in the absence of hypertension than in the presence of it. The relative risk of CAD, except young MI, in normotensives with the DD genotype to (ID + II) was 3.4.

Discussion

Analysis by PCR of ACE insertion/deletion polymorphism in intron 16 demonstrated a statistically significant association of the DD genotype with coronary artery disease. The DD-genotype frequency in not only CAD but also non-

CAD groups was, however, lower than noted in Caucasians[1,2] and in subjects living in the Osaka area, a central part of Japan[16] or in the Morioka area in northern Japan.[17]

Race-related variance in ACE-gene polymorphisms is known. In normal Caucasians, allele D frequency was 0.54–0.58 and was similar in studies reported in Europe,[1] Australia[18] and the United States,[10] while allele D frequency in black Americans was 0.61.[19] In Japanese, allele D-frequency tended to be low and was as variable as 0.27, 0.33, and 0.39 and 0.42 in the present study, Furuya et al,[15] Zhao et al,[20] and Nakai et al,[17] respectively. Since we selected subjects whose parents were residents of the Yamagata area or the northern part of Japan, the present study suggests the importance of ethnic consideration in gene-association studies. The reason for such a wide variability of the D-allele among Japanese or among districts in Japan is unknown.

The D-allele or the DD-genotype is linked significantly to morbidity in cases of myocardial infarction[1,16] and also to the prevalence of restenosis.[21] However, this evidence has become controversial since recent studies have revealed the non-significant association of the D-allele to morbidity in myocardial infarction[22] and to the prevalence of restenosis.[23] The Japanese have a lower incidence of ischemic heart disease,[8] which corresponds in general to a lower frequency of the ACE DD-genotype among the Japanese. However, in populations with a lower frequency of the D-allele, patients with myocardial infarction showed a higher frequency of the D-allele in the Osaka study[16] and in the present study. Accordingly, the association of the ACE genotype to the morbidity of coronary artery disease can be extended to Japanese.

In the present study, the D homozygote in CAD was more prevalent in the non-hypertensive group than in the hypertensive group, a finding which accords well with the original observation that the ACE/DD genotype is a risk factor for coronary artery disease in subjects normally considered to be at a low risk. There were no differences between genotypes for plasma cholesterol level, smoking habits or blood pressure in patients with CAD. Young MI showed similar frequencies of ACE gene polymorphism to those in NCS, a pattern which differed from that seen in subjects with CAD ($p < 0.05$) along with a different risk profile noted in CAD. Thus a study on a candidate gene that relates to the morbidity of coronary artery disease and known risk factors will need to be done to elucidate the pathogenesis and to prevent myocardial infarction.

Acknowledgments

We thank Dr. K. Yokoyama, Yamagata Prefectural Central Hospital, Dr. Y. Takeishi, Ishinomaki Red Cross Hospital and Drs. K. Ikeda, T. Nagai, A. Fukui, N.

Nozaki, O. Hirono, and R. Shiga, Yamagata University Hospital for discussion and assistance in collection of samples from subjects, S. Seino for excellent technical assistance, and A. Funaki and M. Arai for their valuable secretarial services.

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