Effect of Polymorphism of Apolipoprotein E and Angiotensin-Converting Enzyme Genes on Arterial Wall Thickness

Kyoko Kogawa, Yoshiki Nishizawa, Masayuki Hosoi, Takahiko Kawagishi, Kiyoshi Maekawa, Tetsuo Shoji, Yasuhisa Okuno, and Hirotoshi Morii

We examined the association between the polymorphism of the apolipoprotein E (apoE) and the ACE genes and the intima-media thickness (IMT) of the carotid and femoral arteries measured using ultrasonography. The values of IMT of each artery were significantly higher in NIDDM patients (n = 356) than in control subjects (n = 235). The E4 allele or the D allele did not affect clinical characteristics, including age, fasting plasma glucose, total cholesterol, HDL cholesterol, LDL cholesterol, or blood pressure, in NIDDM or control subjects. No difference in the carotid IMT value was noted among the apoE genotypes in control or diabetic subjects. The carotid IMT was significantly higher in diabetic patients with the DD genotype (1.200 ± 0.586 mm) than in those with the II genotypes (0.990 ± 0.364 mm). Neither the E4 allele nor the D allele affected the femoral IMT in control or diabetic subjects. Multiple regression analysis demonstrated that the carotid IMT of NIDDM patients was associated with age, the D allele, and LDL cholesterol but not with the E4 allele, whereas that of control subjects was associated with age, sex, systolic blood pressure, LDL cholesterol, and HDL cholesterol, inversely. These results suggested that the E4 allele was not associated with the carotid or femoral IMTs, but that the D allele was statistically associated with carotid IMT in NIDDM patients but not control subjects. However, since the association was weak (2.3% explanatory power), its biological significance remains to be determined.
***Diabetes* 46:682–687, 1997**

Patients with diabetes have accelerated atherosclerotic vascular lesions. The abnormalities of endothelium, smooth muscle cell, and lipoprotein contribute to the development of atherosclerosis in diabetes (1). In the Framingham study, the risk of vascular diseases including coronary, cerebral, and peripheral arteries was about three times higher in diabetic than in nondiabetic subjects (2).

From the Second Department of Internal Medicine, Osaka City University Medical School, Osaka, Japan.

Address correspondence and reprint requests to Dr. Masayuki Hosoi, Second Department of Internal Medicine, Osaka City University Medical School, 1-5-7, Asahi-machi, Abeno-ku, Osaka 545, Japan.

Received for publication 23 April 1996 and accepted in revised form 7 November 1996.

apoE, apolipoprotein E; CAD, coronary artery disease; IMT, intima-media thickness; PTCA, percutaneous transluminal balloon coronary angioplasty.

Not only metabolic factors but also some genetic factors are thought to be involved in the development of atherosclerosis. The apolipoprotein E (apoE) gene, which is polymorphic and has three common alleles, E2, E3, and E4 (3), is a candidate gene for atherosclerosis. The levels of total and LDL cholesterol have been shown to be higher in subjects with the E4 allele (4). It has been reported that the E4 allele is associated with an increased risk for coronary artery disease (CAD) (4–7) and coronary artery restenosis after percutaneous transluminal balloon coronary angioplasty (PTCA) (8). The differences in lipid metabolism by the apoE genotype and/or the E4 allele itself, which is independent of serum lipid level, have been ascribed to the increased risk of cardiovascular disease. An insertion/deletion polymorphism of ACE affecting the plasma level of this enzyme (9) has been also reported to be associated with myocardial infarction (10) and coronary artery restenosis after PTCA (11). Furthermore, van Bockxmeer et al. (12) showed that the combined carrier state for the D and E4 alleles increased the risk of the coronary artery restenosis.

The extent of carotid atherosclerosis measured using B-mode ultrasonography, a noninvasive method, has been shown to be strongly correlated with the presence of CAD and to be a marker for an early phase of the atherosclerotic process (13). Recently, we reported that the D allele of the ACE gene was the important risk factor of the carotid intima-media thickness (IMT) in NIDDM patients (14).

To clarify the genetic factors relevant to the development of diabetic macroangiopathy, we first examined the association between the polymorphism of the apoE gene and the carotid and femoral IMTs in NIDDM patients. Secondly, we explored the relation between the apoE and the ACE genotypes and arterial wall thickness. Finally, we compared the genetic effect in NIDDM patients with that in nondiabetic control subjects.

RESEARCH DESIGN AND METHODS

Subjects and clinical characteristics. From inpatients and outpatients of Osaka City University Hospital, 356 Japanese patients with NIDDM were recruited. They ranged in age from 18 to 82 years (201 male and 155 female). Inclusion criterion was the presence of ascertained NIDDM, defined as previously reported (15). Two hundred thirty-five nondiabetic control subjects aged 18 to 75 years (85 male and 150 female) were recruited from the participants of a local health check program in the Osaka Municipal Health Promotion Center. Inclusion criteria were systolic blood pressure <160 mmHg and diastolic blood pressure <90 mmHg; fasting plasma glucose <7.8 mmol/l; no history of myocardial infarction, cerebral infarction, or intermittent claudication; and no use of medication. The measurement of blood pressure, the presence of hypertension, and lifelong exposure to smoking were defined as previously described (16).

TABLE 1
Clinical characteristics of control subjects and NIDDM patients

	Control subjects	NIDDM patients
n	235	356
Sex (M/F)	85/150	201/155*
Age (years)	50.9 ± 10.6	59.6 ±10.6*
BMI (kg/m^2)	22.8 ± 2.6	23.1 ± 3.5
Cigarette-years	160.2 ± 298.4	401.6 ± 583.4*
Fasting plasma glucose (mmol/l)	5.4 ± 0.6	9.8 ± 3.0*
Total cholesterol (mmol/l)	5.22 ± 0.81	5.40 ± 1.15*
Triglyceride (mmol/l)	1.30 ± 0.72	1.55 ± 1.21*
HDL cholesterol (mmol/l)	1.58 ± 0.45	1.29 ± 0.39*
LDL cholesterol (mmol/l)	3.09 ± 0.77	3.41 ± 1.05*
Systolic blood pressure (mmHg)	122.0 ± 17.2	137.7 ± 21.9*
Diastolic blood pressure (mmHg)	75.1 ± 11.2	75.3 ± 11.2

Data are *n* or means ± SD. *P < 0.05 vs. control subjects.

Blood was drawn after an overnight fast for the analyses of concentrations of glucose, total cholesterol, triglyceride, HDL cholesterol, HbA_{1c}, and creatinine by standard laboratory methods. LDL cholesterol was estimated by the Friedewald equation (17). Dyslipidemia was defined as previously described (16). In NIDDM patients, the mean values of biochemical parameters and blood pressure obtained three times within a 6-month period were used for statistical analysis. In control subjects, values obtained once within 7 days before ultrasonographic examination were used for statistical analysis. Their clinical and biochemical characteristics are summarized in Table 1. NIDDM patients under ACE inhibitor therapy were excluded from the study.

Ultrasonography. B-mode imaging of the carotid and femoral arteries was performed with high-resolution ultrasonography with a 10-MHz in-line Sectascanner (SSD650CL, Aloka Co. Ltd.), as reported previously (14,16). At each longitudinal projection, the IMT was conducted from the site of the greatest thickness. To assess the intraobserver variability of the recording and measurement of the IMTs in the carotid and femoral arteries, a total of 40 subjects, 20 NIDDM patients and 20 control subjects, were examined on two different occasions. The coefficient of variation for the IMT was as follows: 3.2% for NIDDM patients and 3.6% for control subjects in the carotid artery; and 3.0% for NIDDM patients and 2.8% for control subjects in the femoral artery.

DNA studies. ApoE genotyping was performed by the modified method of Hixon and Vernier (3) from peripheral leukocytes, as previously reported (14). The genotype of the ACE gene was determined by the polymerase chain reaction method according to Rigat et al. (14,18). We confirmed the accuracy of the genotyping results by using an insertion-specific primer pair (5′-CTGGAGACCACTCCCATCCTTTCT-3′ and 5′-TCGAGACCATCCCGGCTAAAAC-3′) to avoid mistyping ID as DD. Only the I allele produced a 290-bp piece. The reaction yields no products in the samples of DD genotype.

Statistical analysis. Data are expressed as means ± SD. Clinical characteristics in control subjects and NIDDM patients were compared using a one-way analysis of variance with Scheffe's F test. Stepwise multiple regression analyses were performed to assess the combined influence of variables on IMT values. First, to examine the effect of risk factors in all subjects (control subjects plus NIDDM patients) on IMT values, the following factors were considered as the independent variables: age; sex (female = 0, male = 1); BMI; cigarette-years; diabetes (absent = 0, present = 1); hypertension (absent = 0, present = 1); dyslipidemia (absent = 0, present = 1); presence of the E4 allele (E2/E2 = 0, E2/E3 = 0, E3/E3 = 0, E2/E4 = 1, E3/E4 = 1, E4/E4 = 1); and presence of the D allele (II = 0, ID = 1, DD = 2). Second, the following factors were considered in control subjects: age, sex, BMI, cigarette-years, fasting plasma glucose, LDL cholesterol, HDL cholesterol, systolic and diastolic blood pressure, E4 allele, and D allele. Lastly, the following factors were considered in NIDDM patients: age, sex, BMI, cigarette-years, duration of diabetes, HbA_{1c}, LDL cholesterol, HDL cholesterol, creatinine, systolic and diastolic blood pressure, E4 allele, and D allele. *F* value to enter was set at 4.0 at each step. A value of $P < 0.05$ was considered statistically significant. These statistical analyses were carried out using Stat View IV (Abacus Concepts, Inc., CA) on a personal computer (Macintosh Centris 650).

RESULTS

Carotid and femoral IMT. Since there was nearly a decade difference between control subjects and NIDDM patients (Table 1), we showed the IMT values in NIDDM patients and age-matched control subjects (Fig. 1). The IMT values of both carotid and femoral arteries in NIDDM patients were significantly greater than those in age-matched control subjects in all age-groups.

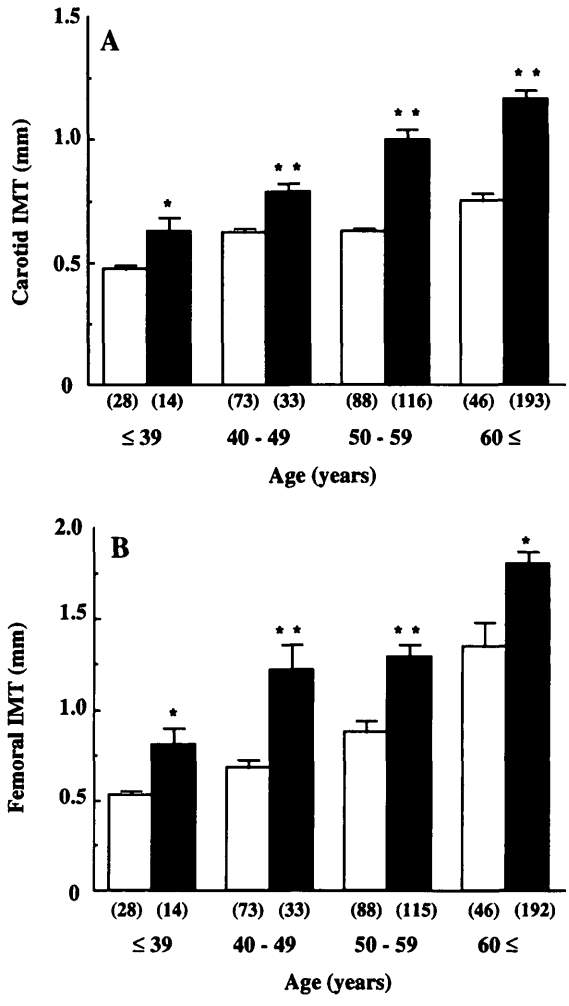

FIG. 1. ***A*: bar graphs showing mean (SE) values of the carotid IMT in control subjects (*n* = 235; □) and NIDDM patients (*n* = 356; ■) according to age. *P < 0.005 vs. control subjects; **P < 0.0001 vs. control subjects. *B*: bar graphs showing mean (SE) values of the femoral IMT in control subjects (*n* = 235; □) and NIDDM patients (*n* = 354; ■) according to age. *P < 0.005 vs. control subjects; **P < 0.0001 vs. control subjects.**

The distributions of apoE and ACE genotypes. The distribution of the apoE genotypes in the patients was similar to that in control subjects (Table 2). There was no significant difference in the distribution of the ACE genotypes between the patients and control subjects by χ^2 test (Table 3). The distributions of the apoE and the ACE genotypes observed were in agreement with the Hardy-Weinberg proportion. The genotype distributions of the apoE and the ACE genes were consistent with other published reports in the Japanese subjects (19,20).

Clinical characteristics among apoE and ACE genotypes. In control subjects, no significant differences among the genotypes of the apoE or the ACE gene were found with respect to clinical characteristics (Table 2, 3). There were no significant differences in any parameters except for triglyceride (1.52 ± 1.06 vs. 1.25 ± 0.63 mmol/l, $P = 0.0362$) between the subjects with the E4 allele (E2/E4, E3/E4, E4/E4) and

TABLE 2
Clinical characteristics of control subjects and NIDDM patients divided by the apoE genotypes

	E2/E2	E2/E3	E3/E3	E2/E4	E3/E4	E4/E4
Control subjects						
n	1 (0.4)	21 (8.9)	176 (74.9)	4 (1.7)	32 (13.6)	1(0.4)
Sex (M/F)	0/1	7/14	68/108	0/4	10/22	0/1
Age (years)	56.0	50.7 ± 11.4	50.7 ± 10.8	50.3 ± 5.0	51.7 ± 10.3	55.0
BMI (kg/m^2)	22.5	22.8 ± 2.7	22.7 ± 2.7	21.6 ± 2.2	23.3 ± 2.5	26.6
Cigarette-years	0	169.3 ± 352.8	156.0 ± 279.9	22.5 ± 45.0	204.7 ± 377.6	0
Fasting plasma glucose (mmol/l)	5.22	5.57 ± 0.74	5.45 ± 0.59	4.87 ± 0.54	5.42 ± 0.64	5.44
Total cholesterol (mmol/l)	5.20	4.92 ± 0.81	5.21 ± 0.79	5.09 ± 1.22	5.48 ± 0.77	6.08
Triglyceride (mmol/l)	2.23	1.23 ± 0.53	1.25 ± 0.64	1.04 ± 0.31	1.57 ± 1.12	175.0
HDL cholesterol (mmol/l)	2.12	1.56 ± 0.45	1.58 ± 0.45	1.86 ± 0.23	1.53 ± 0.48	1.76
LDL cholesterol (mmol/l)	2.06	2.84 ± 0.66	3.10 ± 0.74	2.69 ± 0.99	3.24 ± 0.89	3.41
Systolic blood pressure (mmHg)	130	123.7 ± 21.7	122.0 ± 16.6	117.3 ± 10.4	121.5 ± 18.5	116.0
Diastolic blood pressure (mmHg)	85.0	77.1 ± 12.9	74.9 ± 11.0	76.0 ± 7.0	74.8 ± 12.0	72.0
Carotid IMT (mm)	0.590	0.626 ± 0.176	0.631 ± 0.172	0.610 ± 0.118	0.645 ± 0.149	0.490
Femoral IMT (mm)	0.770	0.760 ± 0.363	0.891 ± 0.629	0.613 ± 0.124	0.875 ± 0.599	0.610
NIDDM patients						
n	—	26 (7.3)	261 (73.3)	7 (2.0)	60 (16.9)	2 (0.6)
Sex (M/F)	—	18/8	146/115	2/5	35/25	0/2
Age (years)	—	59.8 ± 13.0	59.9 ± 10.5	63.9 ± 8.4	57.8 ± 10.2	60.5 ± 0.7
BMI (kg/m^2)	—	23.5 ± 3.5	22.8 ± 3.3	24.4 ± 2.1	24.0 ± 4.0	24.3 ± 2.9
Cigarette-years	—	435.0 ± 470.0	390.7 ± 572.7	0	450.4 ± 669.0	1,330.0 ± 749.5
Duration of diabetes (years)	—	12.2 ± 10.7	11.5 ± 8.5	19.4 ± 12.7	11.3 ± 8.3	18.5 ± 12.0
Fasting plasma glucose (mmol/l)	—	10.2 ± 3.2	9.6 ± 3.0	8.7 ± 2.3	10.5 ± 3.1	8.9 ± 0.6
HbA_{1c} (%)	—	8.6 ± 2.8	8.4 ± 2.3	8.8 ± 2.0	8.9 ± 2.0	7.0 ± 0.5
Total cholesterol (mmol/l)	—	4.88 ± 1.37	5.44 ± 1.13	6.34 ± 1.30	5.33 ± 1.07	5.59 ± 1.24
Triglyceride (mmol/l)	—	1.65 ± 1.09	1.47 ± 0.92	1.46 ± 0.57	1.85 ± 2.09	2.15 ± 0.86
HDL cholesterol (mmol/l)	—	1.18 ± 0.24	1.33 ± 0.41	1.32 ± 0.52	1.17 ± 0.28	1.01 ± 0.26
LDL cholesterol (mmol/l)	—	2.95 ± 1.12	3.44 ± 1.05	4.35 ± 1.47*	3.33 ± 0.88	3.59 ± 0.59
Creatinine (μmol/l)	—	73.4 ± 37.1	84.0 ± 100.8	70.7 ± 38.0	85.7 ± 103.4	134.4 ± 114.0
Systolic blood pressure (mmHg)	—	135.6 ± 21.2	137.7 ± 21.2	151.4 ± 7.2	136.7 ± 26.3	151.5 ± 3.5
Diastolic blood pressure (mmHg)	—	71.9 ± 10.5	75.5 ± 11.3	85.7 ± 4.7	74.9 ± 11.0	75.5 ± 3.5
Carotid IMT (mm)	—	0.993 ± 0.495	1.043 ± 0.485	1.378 ± 0.623	1.098 ± 0.490	1.025 ± 0.063
Femoral IMT (mm)	—	1.340 ± 0.809	1.594 ± 0.901	2.110 ± 0.868	1.314 ± 0.751	2.332 ± 0.996

Data are *n* (%) or means ± SD. *P < 0.05 vs. patients with the E2/E3 genotype.

the subjects without the E4 allele (E2/E2, E2/E3, E3/E3). In NIDDM patients, no significant differences among the genotypes of the apoE or the ACE gene were found with respect to clinical characteristics except for LDL cholesterol (Tables 2 and 3). Serum LDL cholesterol was higher in the patients with E2/E4 compared with the values in the patients with E2/E3. There were no significant differences in any parameters except for BMI (24.1 ± 3.8 vs. 22.9 ± 3.4, P = 0.0132) or triglyceride (1.82 ± 1.96 vs. 1.49 ± 0.94 mmol/l, P = 0.0427) between the patients with the E4 allele and the patients without the E4 allele.

Relationships between apoE and ACE genotypes and IMT. In control subjects, there were no significant differences in the carotid IMT either among the apoE genotypes or among the ACE genotypes (Tables 2 and 3). No significant differences were found in the carotid IMT between the patients with the E4 allele and those without the E4 allele. On the other hand, the carotid IMT was significantly higher in NIDDM patients with the DD genotype (1.200 ± 0.586 mm) than in those with the II genotype (0.990 ± 0.364 mm) (Table 3). The carotid IMT of the patients with the D-present genotypes (ID plus DD) (1.101 ± 0.557 mm) was also higher than that with the D-absent genotype (II) (0.991 ± 0.362 mm). There were no significant differences in the femoral IMT among the apoE genotypes or the ACE genotypes in control or NIDDM subjects (Tables 2 and 3).

Effect of diabetic state on carotid and femoral IMT. Multiple regression analysis in all subjects demonstrated that the carotid IMT was associated with age, the presence of diabetes, the D allele, sex, and hypertension (Table 4), while the femoral IMT was associated with age, the presence of diabetes, sex, hypertension, dyslipidemia, and cigarette smoking (Table 4).

Risk factors significantly related to carotid and femoral IMT in control subjects and NIDDM patients. Multiple regression analysis was done in control or diabetic subjects to determine relationships between the IMT and possible risk factors. In control subjects, age, sex, systolic blood pressure, LDL cholesterol, and HDL cholesterol were independently associated with the carotid IMT (Table 4). Age and cigarette smoking were independently associated with the femoral IMT (Table 4). In NIDDM patients, age, the D allele, and LDL cholesterol were independently associated with the carotid IMT (Table 4). The risk factors associated with the femoral IMT were age, systolic blood pressure, sex, and duration of diabetes (Table 4).

Relationships among IMT and apoE and ACE genotypes. The IMT values of the carotid artery tended to be greater in NIDDM patients with the E4 allele than in patients

TABLE 3
Clinical characteristics of control subjects and NIDDM patients grouped by ACE genotypes

	Control subjects			NIDDM patients		
	II	ID	DD	II	ID	DD
n	87 (37.0)	116 (49.4)	32 (13.6)	147 (41.3)	149 (41.9)	60 (16.9)
Sex (M/F)	33/54	43/73	9/23	78/69	92/57	31/29
Age (years)	51.4 ± 11.5	51.5 ± 10.4	46.9 ± 7.9	60.2 ± 9.5	58.8 ± 11.8	60.0 ± 9.6
BMI (kg/m^2)	22.8 ± 2.8	22.7 ± 2.5	22.8 ± 2.9	22.9 ± 3.0	23.2 ± 3.7	23.5 ± 4.0
Cigarette-years	137.5 ± 284.0	163.8 ± 289.3	209.4 ± 365.7	408.4 ± 542.7	418.9 ± 638.4	342.2 ± 540.0
Duration of diabetes (years)	—	—	—	11.3 ± 8.7	12.0 ± 8.9	12.3 ± 8.6
Fasting plasma glucose (mmol/l)	5.5 ± 0.7	5.4 ± 0.5	5.4 ± 0.6	9.8 ± 3.0	10.0 ± 3.3	9.1 ± 2.5
HbA_{1c} (%)	—	—	—	8.5 ± 2.2	8.5 ± 2.4	8.2 ± 2.0
Total cholesterol (mmol/l)	5.17 ± 0.86	5.21 ± 0.74	5.44 ± 0.87	5.31 ± 1.28	5.45 ± 1.02	5.51 ± 1.10
Triglyceride (mmol/l)	1.33 ± 0.70	1.23 ± 0.63	1.44 ± 1.05	1.56 ± 1.50	1.54 ± 0.91	1.56 ± 1.19
HDL cholesterol (mmol/l)	1.57 ± 0.49	1.58 ± 0.43	1.60 ± 0.42	1.31 ± 0.40	1.28 ± 0.38	1.27 ± 0.37
LDL cholesterol (mmol/l)	3.04 ± 0.78	3.10 ± 0.72	3.17 ± 0.89	3.30 ± 1.22	3.47 ± 0.89	3.53 ± 0.93
Creatinine (μmol/l)	—	—	—	79.6 ± 114.9	88.4 ± 88.4	88.4 ± 88.4
Systolic blood pressure (mmHg)	122.9 ± 17.7	121.9 ± 17.3	119.8 ± 15.2	137.0 ± 22.5	137.5 ± 22.5	139.9 ± 19.0
Diastolic blood pressure (mmHg)	75.6 ± 11.5	74.9 ± 11.2	74.4 ± 10.7	74.7 ± 10.9	75.8 ± 11.1	75.5 ± 11.8
Carotid IMT (mm)	0.629 ± 0.162	0.631 ± 0.171	0.640 ± 0.173	0.990 ± 0.364	1.062 ± 0.541	1.200 ± 0.586*
Femoral IMT (mm)	0.915 ± 0.647	0.887± 0.608	0.691 ± 0.357	1.466 ± 0.800	1.540 ± 0.928	1.735 ± 0.928

Data are *n* (%) or means ± SD. *$P < 0.05$ vs. patients with the II genotype.

without the E4 allele. The carotid IMT of NIDDM patients with both the E4- and D-present genotypes (1.150 ± 0.574 mm) was greater ($P = 0.23$, not significant) than that without the E4 or D genotypes (0.974 ± 0.369 mm).

DISCUSSION

We demonstrated that the values of IMT of the carotid and femoral arteries were significantly higher in NIDDM patients than in age-matched control subjects, suggesting that atherosclerosis was accelerated in diabetes, as previous studies also suggest (21,22).

Nondiabetic subjects with CAD carrying the E4 allele are reported to have higher levels of total cholesterol and LDL cholesterol than do patients homozygous for the E3 allele (4). But in NIDDM patients, there was no difference in total cholesterol or LDL cholesterol among the apoE genotypes (20). This may be the reason for the failure to show a correlation between the apoE genotype and serum lipid level in our study. The presence of the E4 allele is associated with an increased risk of CAD through the effect of increased total and LDL cholesterol caused by the E4 allele (4,23) and/or a genetic effect independent of the effect of serum lipid level (6,7,24). On the other hand, Marshall et al. (25) found no evidence of a correlation between the apoE polymorphism and angiographically-defined CAD. Our study showed that the E4 allele was not a risk factor for the carotid or femoral IMTs in control subjects or NIDDM patients.

Multiple regression analysis was conducted to assess the independent effects of the different risk factors. In NIDDM patients, age, the D allele, and LDL cholesterol were determinants of the carotid IMT, explaining 11.7, 2.3, and 1.4% of the variance of the IMT, respectively. Although the D allele was statistically associated with the carotid IMT in diabetic patients, the D allele may not be biologically important, because the D allele added an only 2.3% explanatory power in NIDDM patients. Interestingly, the percentage of the variance of the carotid IMT in NIDDM explained by the D allele was as much as that explained by LDL cholesterol, a traditional biological risk factor for atherosclerosis.

The D allele statistically correlated with the carotid IMT in NIDDM patients but not in control subjects. The explanatory power of the D allele was slightly greater in NIDDM patients (2.3%) than in all subjects (1.4%). Although the mechanism(s) remain still unclear, our data suggest that D allele might be a gene in determining the values of the carotid IMT when exposed to diabetic state. Such a gene-environment interaction is pointed out in the polymorphism of β-fibrinogen (26) and glycogen synthase gene (27). On the other hand, Castellano et al. (28) reported that the DD genotype in the general population in Italy was associated with the increased carotid IMT. The fact that the frequency of the DD genotype in Italy is higher than that in Japan (28) may explain the discrepancy between the Italian study and our study.

The predictors of femoral IMT in all subjects were age, diabetes, hypertension, sex, dyslipidemia, and smoking. Age, diabetes, hypertension, and sex were associated with the IMTs of both arteries. Neither the E4 allele nor the D allele affected the femoral IMT in diabetic or control subjects. In autopsy studies, the relationship between coronary and femoral atherosclerosis was weak (29). Atherosclerotic changes occur more often in curved arteries than in straight arteries (30). Taken together, it may be conceivable that different artery segments are affected to a different degree by other factors such as hemodynamic factors, properties of the arteries, and/or genetic ones.

van Bockxmeer et al. (12) suggested that the combined carrier state for the E4 and D alleles increased restenosis after PTCA. In our study, although the carotid IMT tended to be greater in patients with both the E4 and D alleles than in patients with neither the E4 nor D alleles, we could not find a significant interaction between the apoE and the ACE genotypes.

The DD genotype is associated with the increase in the ACE contents in T-lymphocytes (31) and cardiac tissue (32), as well

TABLE 4
Risk factors affecting the IMTs of the carotid and femoral arteries in all subjects, control subjects, and NIDDM patients

Dependent variable	All subjects			Control subjects			NIDDM patients		
	Independent variables	β	*F* value	Independent variables	β	*F* value	Independent variables	β	*F* value
Carotid IMT	Age	0.322	73.430	Age	0.430	53.623	Age	0.355	49.799
	Diabetes	0.298	55.653	Sex	0.177	7.884	D allele	0.143	8.077
	D allele	0.117	11.775	Systolic blood pressure	0.160	7.735	LDL cholesterol	0.119	5.564
	Sex	0.080	5.222	LDL cholesterol	0.155	6.983			
	Hypertension	0.084	5.033	HDL cholesterol	−0.122	4.215			
	$R^2 = 0.327$ ($P < 0.0001$)			$R^2 = 0.297$ ($P < 0.0001$)			$R^2 = 0.154$ ($P < 0.0001$)		
Femoral IMT	Age	0.366	92.337	Age	0.412	49.405	Age	0.293	33.517
	Diabetes	0.141	11.786	Smoking	0.225	14.787	Systolic blood pressure	0.215	18.211
	Hypertension	0.114	8.967				Sex	0.184	14.285
	Sex	0.104	6.703				Duration of diabetes	0.164	10.592
	Dyslipidemia	0.089	6.119						
	Smoking	0.098	5.900						
	$R^2 = 0.314$ ($P < 0.0001$)			$R^2 = 0.214$ ($P < 0.0001$)			$R^2 = 0.233$ ($P < 0.0001$)		

Significant predictors of the carotid and femoral IMT in all subjects were explored among the parameters, including age, sex (female = 0, male = 1), BMI, cigarette-years, diabetes (absent = 0, present = 1), hypertension (absent = 0, present = 1), dyslipidemia (absent = 0, present = 1), E4 allele (absent = 0, present = 1), and D allele (II = 0, ID = 1, DD = 2). In control subjects, the following factors were explored: age, sex, BMI, cigarette-years, fasting plasma glucose, LDL cholesterol, HDL cholesterol, systolic and diastolic blood pressure, E4 allele, and D allele. In NIDDM patients, the following factors were explored: age, sex, BMI, cigarette-years, duration of diabetes, HbA_{1c}, LDL cholesterol, HDL cholesterol, serum creatinine, systolic and diastolic blood pressure, E4 allele, and D allele. *F* value to enter was set at 4.0 at each step. β is standard regression coefficient; R^2 is multiple coefficient of determination.

as the circulating level (9). Although whether the plasma ACE molecule comes only from vascular endothelial cells is still unknown to date (33), it is tempting to speculate that the genetic control or ACE insertion/deletion polymorphism is exerted at regulating the vascular ACE expression. Cooper et al. (34) reported that diabetes was associated with increased mesenteric ACE levels and wall thickness in streptozocin-induced diabetic rats, and that ACE inhibitor attenuated this vascular hypertrophy. Bonithon-Kopp et al. (35) showed that plasma ACE activity was significantly high in subjects with increased carotid IMT. Thus, in NIDDM patients, especially those with the D allele, the plasma and/or vascular ACE activity might be high, which might be relevant to the accelerated atherosclerosis.

In summary, the E4 allele did not affect the carotid or femoral IMTs in control or diabetic subjects. Dividing all subjects into NIDDM patients and control subjects, the D allele was statistically associated with carotid IMT only in NIDDM patients. However, since the D allele explained just <3% of the carotid IMT variance, it might not be a biologically significant determinant for the carotid IMT. A longitudinal study on the relation between the ACE genotype and the change in the carotid IMT is necessary to draw conclusions on this issue.

ACKNOWLEDGMENTS

The authors are grateful to Keiko Kawanishi and Mayuko Murano for the expert technical assistance and to Kyoko Izumotani, the Osaka Municipal Health Promotion Center, for obtaining blood samples from subjects. This work was supported in part by grants from the Japanese Ministry of Education, Science, and Culture and from the Japan Owner's Association.

This study was presented in part in abstract form at the 56th annual meeting of the American Diabetes Association, San Francisco, CA, 1996.

REFERENCES

1. Colwell JA, Lopes-Virella M, Halushka PV: Pathogenesis of atherosclerosis in diabetes mellitus. *Diabetes Care* 4:121–133, 1981
2. Garcia MJ, McNamara PM, Gordon T, Kannell WB: Morbidity and mortality in diabetics in the Framingham population: sixteen year follow-up study. *Diabetes* 23:105–111, 1973
3. Hixson JE, Vernier DT: Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res* 31:545–548, 1990
4. Lenzen HJ, Assmann G, Buchwalsky R, Schulte H: Association of apolipoprotein E polymorphism, low-density lipoprotein cholesterol, and coronary artery disease. *Clin Chem* 32:778–781, 1986
5. van Bockxmeer F, Mamotte CD: Apolipoprotein epsilon 4 homozygosity in young men with coronary heart disease. *Lancet* 340:879–880, 1992
6. Eichner JE, Kuller LH, Orchard TJ, Grandits GA, McCallum LM, Ferrell RE, Neaton JD: Relation of apolipoprotein E phenotype to myocardial infarction and mortality from coronary artery disease. *Am J Cardiol* 71:160–165, 1993
7. Wang XL, McCredie RM, Wilcken DE: Polymorphisms of the apolipoprotein E gene and severity of coronary artery disease defined by angiography. *Arterioscler Thromb Vasc Biol* 15:1030–1034, 1995
8. van Bockxmeer F, Mamotte CD, Gibbons FR, Taylor RR: Apolipoprotein epsilon 4 homozygosity—a determinant of restenosis after coronary angioplasty. *Atherosclerosis* 110:195–202, 1994
9. Rigat B, Hubert C, Alhenc GF, Cambien F, Corvol P, Soubrier F: An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 86:1343–1346, 1990
10. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, Tiret L, Amouyel P, Alhenc-Gelas F, Soubrier F: Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk

factor for myocardial infarction. *Nature* 359:641–644, 1992
11. Ohishi M, Fujii K, Minamino T, Higaki J, Kamitani A, Rakugi H, Zhao Y, Mikami H, Miki T, Ogihara T: A potent genetic risk factor for restenosis. *Nature Genet* 5:324–325, 1993
12. van Bockxmeer F, Mamotte CD, Gibbons FA, Burke V, Taylor RR: Angiotensin-converting enzyme and apolipoprotein E genotypes and restenosis after coronary angioplasty. *Circulation* 92:2066–2071, 1995
13. Wofford J, Kahl F, Howard G, McKinney W, Toole J, Crouse JI: Relation of extent of extracranial carotid artery atherosclerosis as measured by B-mode ultrasound to the extent of coronary atherosclerosis. *Arterioscler Thromb* 11:1786–1794, 1991
14. Hosoi M, Nishizawa Y, Kogawa K, Kawagishi T, Konishi T, Maekawa K, Emoto M, Fukumoto S, Shioi A, Shoji T, Inaba M, Okuno Y, Morii H: Angiotensin converting enzyme gene polymorphism is associated with carotid arterial wall thickness in non-insulin-dependent diabetic patients. *Circulation* 94:704–707, 1996
15. Ruiz J, Blanche H, Cohen N, Velho G, Cambien F, Cohen D, Passa P, Froguel P: Insertion/deletion polymorphism of the angiotensin-converting enzyme gene is strongly associated with coronary heart disease in non-insulin-dependent diabetes mellitus. *Proc Natl Acad Sci USA* 91:3662–3665, 1994
16. Kawagishi T, Nishizawa Y, Konishi T, Kawasaki K, Emoto M, Shoji T, Tabata T, Inoue T, Morii H: High-resolution B-mode ultrasonography in evaluation of atherosclerosis in uremia. *Kidney Int* 48:820–826, 1995
17. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low lipoprotein in plasma without the use of preparative ultracentrifuge. *Clin Chem* 18:499–502, 1972
18. Rigat B, Hubert C, Corvol P, Soubrier F: PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). *Nucleic Acids Res* 20:1433, 1992
19. Fujisawa T, Ikegami H, Shen G-Q, Yamato E, Takekawa E, Nakagawa Y, Hamada Y, Ueda H, Rakugi H, Higaki J, Ohishi M, Fujii K, Fukuda M, Ogihara T: Angiotensin I- converting enzyme gene polymorphism is associated with myocardial infarction, but not with retinopathy or nephropathy, in NIDDM. *Diabetes Care* 18:983–985, 1995
20. Eto M, Watanabe K, Iwashima Y, Morikawa A, Oshima E, Sekiguchi M, Ishii K: Apolipoprotein E polymorphism and hyperlipemia in type II diabetics. *Diabetes* 35:1374–1382, 1986
21. Yamasaki Y, Kawamori R, Matsushima H, Nishizawa H, Kodama M, Kajimoto Y, Morishima T, Kamada T: Atherosclerosis in carotid artery of young IDDM patients monitored by ultrasound high-resolution B-mode imaging. *Diabetes* 43:634–639, 1994
22. Pujia A, Gnasso A, Irace C, Colonna A, Mattioli PL: Common carotid arterial wall thickness in NIDDM subjects. *Diabetes Care* 17:1330–1336, 1994
23. Davignon J, Gregg RE, Sing CF: Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis* 8:1–21, 1988
24. Luc G, Bard JM, Arveiler D, Evans A, Cambou JP, Bingham A, Amouyel P, Schaffer P, Ruidavets JB, Cambien F, Fruchart JC, Ducimetiere P: Impact of apolipoprotein E polymorphism on lipoproteins and risk of myocardial infarction: the ECTIM Study. *Arterioscler Thromb* 14:1412–1419, 1994
25. Marshall HW, Morrison LC, Wu LL, Anderson JL, Corneli PS, Stauffer DM, Allen A, Karagounis LA, Ward RH: Apolipoprotein polymorphisms fail to define risk of coronary artery disease: results of a prospective, angiographically controlled study. *Circulation* 89:567–577, 1994
26. Humphries SE, Ye S, Talmud P, Bara L, Wilhelmsen L, Tiret L: European atherosclerosis research study: genotype at the fibrinogen locus (G_455-A β-gene) is associated with differences in plasma fibrinogen level in young men and women from different regions in Europe: evidence for sex-genotype-environment interaction. *Arterioscler Thromb Vasc Biol* 15:96–104, 1995
27. Schalin-Jantti C, Nikula-Ijas P, Huang X, Lehto M, Knudsen P, Syvanne M, Lehtovirta MT, Tikkanen I, Groop LC: Polymorphism of the glycogen synthase gene in hypertensive and normotensive subjects. *Hypertension* 27:67–71, 1996
28. Castellano M, Muiesan M, Rizzoni D, Beschi M, Pasini G, Cinelli A, Salvetti M, Porteri E, Betoni G, Kreutz R, Lindpaintner K, Rosei E: Angiotensin-converting enzyme I/D polymorphism and arterial wall thickness in a general population: the Vobarno study. *Circulation* 91:2721–2724, 1995
29. Sternby N: Atherosclerosis in a defined population: an autopsy survey in Malmø, Sweden. *Acta Pathol Microbiol Scand* 194 (Suppl. I):111–118, 1968
30. Wendelhag I, Wiklund O, Wikstrand J: Atherosclerotic changes in the femoral and carotid arteries in familial hypercholesterolemia: ultrasonographic assessment of intima-media thickness and plaque occurrence. *Arterioscler Thromb* 13:1404–1411, 1993
31. Costerousse O, Allegrini J, Lopez M, Alhenc-Gelas F: Angiotensin I-converting enzyme in human circulating mononuclear cells: genetic polymorphism of expression in T-lymphocytes. *Biochem J* 290:33–40, 1993
32. Danser A, Schalekamp M, Bax W, van den Brink A, Saxena P, Riegger G, Schunkert H: Angiotensin-converting enzyme in the human heart: effect of the deletion/insertion polymorphism. *Circulation* 92:1387–1388, 1995
33. Johnston C: Tissue angiotensin converting enzyme in cardiac and vascular hypertrophy, repair, and remodeling. *Hypertension* 23:258–268, 1994
34. Cooper M, Rumble J, Komers R, He-Cheng D, Jandeleit K, Sheung-To C: Diabetes-associated mesenteric vascular hypertrophy is attenuated by angiotensin-converting enzyme inhibition. *Diabetes* 43:1221–1228, 1994
35. Bonithon KC, Ducimetiere P, Touboul PJ, Feve JM, Billaud E, Courbon D, Heraud V: Plasma angiotensin-converting enzyme activity and carotid wall thickening. *Circulation* 89:952–954, 1994