Association of ACE Gene Polymorphism With Arterial Stiffness in Patients With Type 2 Diabetes

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OBJECTIVE — To assess the relationship between the insertion (I)/deletion (D) polymorphism of the ACE gene and arterial distensibility in patients with type 2 diabetes and healthy control subjects.

RESEARCH DESIGN AND METHODS — Aortic and carotid arterial distensibility were evaluated by measuring aortic pulse-wave velocity (a-PWV) and carotid stiffness β using an echo-tracking system in 137 patients with type 2 diabetes and 260 age-matched control subjects.

RESULTS — a-PWV and carotid stiffness β were significantly higher in patients with type 2 diabetes than in age-matched control subjects ($P < 0.05$). Both stiffness β and a-PWV were significantly higher in the patients with the II genotype than in those with the DD genotype ($P < 0.001$). In the control subjects, multiple regression analysis showed that age and decreased HDL cholesterol were independently associated with increased a-PWV ($R^2 = 0.244$, $P < 0.0001$) and that age, systolic and diastolic blood pressure, and BMI were independently associated with increased carotid stiffness β ($R^2 = 0.454$, $P < 0.0001$). In the patients with type 2 diabetes, age, gene dose of the I allele, and systolic and diastolic blood pressure were independently associated with increased a-PWV ($R^2 = 0.545$, $P < 0.0001$), and age, gene dose of the I allele, and systolic blood pressure were associated with increases in carotid stiffness β ($R^2 = 0.314$, $P < 0.0001$).

CONCLUSIONS — These results suggested that ACE polymorphism is associated with the impairment of aortic and carotid distensibility in patients with type 2 diabetes.

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Type 2 diabetes is associated with an excessively high rate of mortality and morbidity due to macrovascular diseases (1–6). However, the mechanism underlying the macrovascular disease is not clear. It has been suggested that the progression of macrovascular disease could begin before the onset of clinical diabetes (7). The occurrence of such diseases cannot be fully explained by metabolic factors related to diabetes such as hyperglycemia, hypertension, or hyperlipidemia. Some genetic factors are considered to be involved in the development of vascular complications of type 2 diabetes.

An insertion (I)/ deletion (D) polymorphism in intron 16 of the ACE gene is associated with myocardial infarction in some populations (8) as well as type 2 diabetic patients (9). Associations with ischemic heart disease (10), coronary atherosclerosis, dilated cardiomyopathy (11), coronary artery restenosis (12), and cardiac hypertrophy (13) have also been reported. However, these studies yielded conflicting resells: some found positive associations with ACE genotype, and others did not.

There are two components of atherosclerosis: atherosis (morphologic wall thickening) and sclerosis (functional stiffening) of the arterial wall (14). It has been established that the extent of carotid atherosclerosis (intima-media thickness [IMT]) is a marker for an early phase of the atherosclerotic process (15). However, IMT only provides information about vessel wall anatomy. Other important aspects of atherosclerosis related to vessel function, such as wall composition or stiffness, can also be studied in diabetic subjects with other noninvasive techniques (16). Several studies have indicated decreased distensibility of the large arteries of patients with diabetes (17,18). Distensibility of the large arteries can be assessed by measuring the thoracic-abdominal pulse-wave velocity, recorded as aortic pulse-wave velocity (a-PWV) (19). One study demonstrated that carotid arterial stiffness was associated with morphologic changes (20), whereas another suggested that it is relatively independent of IMT (21). In animal studies, a direct relationship has been established between regression of atherosclerosis and an increase in arterial distensibility (22).

We therefore assessed ACE insertion/deletion polymorphisms and the stiffness of the common carotid artery and the thoraco-abdominal aorta in patients with type 2 diabetes and control subjects to identify any association between ACE genotype and sclerotic changes.

RESEARCH DESIGN AND METHODS

Subjects and clinical characteristics

The subjects included 137 Japanese patients with type 2 diabetes (23), 72 men and 65 women, 18 to 75 years old. Patients taking ACE inhibitors and with evidence of cardiovascular disease were excluded. As controls, 260 nondiabetic subjects (18–75 years old) with systolic blood pressure <160 mmHg

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Abbreviations: a-PWV, aortic pulse-wave velocity; I/D, insertion/deletion; IMT, intima-media thickness.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical characteristics of patients with type 2 diabetes and control subjects*

	Diabetic patients	Control subjects
n	137	260
Sex (M/F)	72/65	159/101
Age (years)	53.0 ± 13.7	50.6 ± 11.6
BMI (kg/m²)	22.8 ± 3.5	22.6 ± 2.8
Cigarette-years	490.7 ± 679.5*	198.0 ± 388.0
Duration of diabetes (years)	9.3 ± 8.2	
Fasting plasma glucose (mmol/l)	10.6 ± 4.0*	5.4 ± 0.6
HbA_{1c} (%)	9.5 ± 2.6	
Total cholesterol (mmol/l)	5.30 ± 1.35	5.13 ± 0.89
Triglycerides (mmol/l)	1.41 ± 0.71	1.30 ± 0.74
HDL cholesterol (mmol/l)	1.28 ± 0.45*	1.52 ± 0.46
LDL cholesterol (mmol/l)	3.36 ± 1.20*	3.04 ± 0.79
Systolic blood pressure (mmHg)	133.9 ± 22.1*	122.1 ± 18.0
Diastolic blood pressure (mmHg)	72.9 ± 11.4*	74.4 ± 11.8
Therapy (diet/sulfonylurea/insulin)	36/74/27	
a-PWV (m/s)		
Total	9.96 ± 2.82†	7.40 ± 1.28
≤39 years	6.84 ± 0.98*	6.32 ± 0.70
40–49 years	9.18 ± 1.65*	7.22 ± 0.85
50–59 years	10.34 ± 2.94*	7.40 ± 1.08
≥60 years	11.35 ± 2.44*	8.35 ± 1.68
Carotid stiffness β		
Total	15.0 ± 7.2†	10.9 ± 3.8
≤39 years	8.37 ± 2.84*	7.12 ± 2.15
40–49 years	14.12 ± 11.01*	9.20 ± 2.37
50–59 years	15.49 ± 5.38*	11.61 ± 15.49
≥60 years	17.94 ± 6.46*	14.27 ± 4.08

Data are means ± SD. *$P < 0.05$; †$P < 0.001$ vs. control subjects.

and diastolic blood pressure <90 mmHg; serum fasting plasma glucose <7.8 mmol/l; no history of myocardial infarction, cerebral infarction, or peripheral vascular disease; and not currently on any medication were also examined. The experimental design was approved by our institutional ethics committee, and all patients gave their informed consent to participate.

Blood pressure was determined with a standard mercury sphygmomanometer and cuffs adapted to arm circumference after the subject had rested for at least 15 min. Systolic blood pressure was taken as the point of appearance of Korotkoff sounds and the diastolic blood pressure as the point of disappearance of the sounds. Results are reported as averages of three measurements. Hypertension was defined as *1*) the taking of antihypertensive agents or a history of this disorder, *2*) a systolic blood pressure >160 mmHg, or *3*) a diastolic blood pressure >95 mmHg.

Information on smoking habit was obtained with a self-administered questionnaire. Life-long exposure to smoking was estimated as the product of years smoked and the number of tobacco products smoked daily at the time of the study. The product was used in statistical analysis as cigarette-years.

Blood was drawn after an overnight fast for analysis of serum concentrations of glucose, total cholesterol, triglycerides, HDL cholesterol, and HbA_{1c} by standard laboratory methods. LDL cholesterol was estimated by the Friedewald equation (24). Patients were considered dyslipidemic if they were taking antihyperlipidemic agents or serum cholesterol was >5.69 mmol/l (220 mg/dl), HDL cholesterol was <1.03 mmol/l (40 mg/dl), or triglycerides were >1.70 mmol/l (150 mg/dl), according to the criteria of the Japan Atherosclerosis Society (25).

In patients with type 2 diabetes, the mean values of biochemical parameters and blood pressure obtained three times over 6 months were used for statistical analysis. In control subjects, biochemical parameters and blood pressure were obtained at the same time as measurement of a-PWV and stiffness β and used for statistical analysis. The clinical and biochemical characteristics of patients with type 2 diabetes and control subjects are summarized in Table 1.

Measurement of a-PWV

a-PWV was measured in the supine position after 15 min of bed rest (26) using a pulse-wave velocimeter (model PWV-200; Fukuda Denshi, Tokyo). Briefly, amorphous sensors were placed on the skin at the right femoral and left carotid arteries to record pulse waves. Heart sounds S1 and S2 were detected with a microphone set on the right edge of the sternum at the second intercostal space. Electrocardiogram was monitored with electrodes placed on the right and left arms and the right leg. The PWV meter measured the time intervals between pulse waves at carotid and femoral sites (T) and between S2 and the notch of the carotid pulse wave (Tc). PWV of the aorta (m/s) was calculated as 1.3 L/(T + Tc), where L is the measured distance between the carotid and femoral probes. The actual distance between the aortic orifice and the femoral site was calculated as 1.3L (27). Since (T + Tc), the time for the pulse waves to travel from the aortic orifice to the femoral artery, is dependent on blood pressure, the PWV values were standardized for a diastolic pressure of 80 mmHg. PWV was measured for five consecutive pulses, and averages were used for analysis. The coefficients of variation for a-PWV were 4.2% for patients with type 2 diabetes and 4.5% for control subjects.

Ultrasonographic measurements of arterial distensibility

Vessel diameter and changes in pulsatile diameter were measured by echo-tracking sonography (28) using a recently developed ultrasound echo-tracking system capable of detecting vessel wall movements of less than 10 μm (29). The instrument consists of an electronic echo-tracking instrument interfaced with a real-time ultrasound scanner and fitted with a 7.5-MHz linear array transducer (Aloka SSD610; Aloka, Tokyo). Briefly, two electronic markers automatically lock to the luminal interface of echoes from the anterior and posterior vessel wall and follow the pulsatile movements of the vessel wall. The markers are shown in real time to indicate the level at which the registration is performed. In this system, the smallest detectable movement is 10 μm (29).

Table 2—*Clinical characteristics of patients with type 2 diabetes and control subjects by ACE genotype*

	Patients with type 2 diabetes			Control subjects		
	II	ID	DD	II	ID	DD
n (%)	59 (43.1)	61 (44.5)	17 (12.4)	101 (38.8)	133 (51.2)	26 (10.0)
Sex (M/F)	32/27	34/27	11/6	63/38	78/55	18/8
Age (years)	52.6 ± 11.4	54.0 ± 15.8	51.2 ± 13.9	50.4 ± 12.7	51.2 ± 11.2	48.2 ± 8.7
BMI (kg/m^2)	22.6 ± 3.1	22.9 ± 3.8	23.1 ± 4.2	22.7 ± 2.3	22.5 ± 2.6	22.7 ± 2.3
Cigarette-years	508 ± 686	442 ± 526	364 ± 359	224 ± 379	213 ± 436	172 ± 319
Duration of diabetes (years)	9.8 ± 8.1	9.6 ± 8.8	6.2 ± 5.5			
Fasting plasma glucose (mmol/l)	10.7 ± 4.1	10.6 ± 4.1	10.4 ± 3.8	5.4 ± 0.6	5.3 ± 0.5	5.3 ± 0.5
HbA_{1c} (%)	9.8 ± 2.4	9.3 ± 2.7	9.2 ± 3.3			
Total cholesterol (mmol/l)	5.34 ± 1.06	5.24 ± 1.60	5.34 ± 1.32	5.14 ± 0.92	5.11 ± 0.89	5.27 ± 0.99
Triglycerides (mmol/l)	1.4 ± 0.65	1.31 ± 0.51	1.76 ± 1.28	1.35 ± 0.73	1.24 ± 0.64	1.39 ± 1.15
HDL cholesterol (mmol/l)	1.34 ± 0.49	1.23 ± 0.40	1.27 ± 0.45	1.51 ± 0.49	1.52 ± 0.44	1.57 ± 0.43
LDL cholesterol (mmol/l)	3.35 ± 1.04	3.40 ± 1.39	3.26 ± 0.99	3.04 ± 0.82	3.04 ± 0.77	3.05 ± 0.86
Systolic blood pressure (mmHg)	134 ± 23	133 ± 22	138 ± 23	122 ± 18	123 ± 18	124 ± 18
Diastolic blood pressure (mmHg)	73 ± 11	72 ± 11	78 ± 15			
a-PWV (m/s)	10.90 ± 2.98*	9.56 ± 2.63	8.11 ± 1.34	7.40 ± 1.30	7.50 ± 1.28	6.87 ± 1.20
Carotid stiffness β	17.3 ± 8.3*	13.6 ± 6.0	12.1 ± 4.5	10.4 ± 3.9	11.3 ± 3.8	10.5 ± 3.4

Data are means ± SD. *$P < 0.05$ vs. patients with the DD or ID genotype.

The distensibility of the arterial walls was calculated as follows:

$$\text{stiffness } (\beta) = \ln(P_{syst}/P_{diast})/(D_{syst} - D_{diast})/D_{diast}$$

where P_{syst} and P_{diast} are the maximal systolic and end diastolic blood pressure levels (mmHg), respectively, and D_{syst} and D_{diast} are the corresponding vessel diameters (mm). Each subject was examined three times at each location. The coefficient of variation for carotid artery stiffness was 3.6% for the patients with type 2 diabetes and 3.9% for control subjects.

Genotype investigations

The ACE I/D polymorphism was determined by polymerase chain reaction amplification of ACE DNA (30,31). We confirmed the accuracy of the genotyping results using an insertion-specific primer pair (5′-CTGGAGACCACTCCCA TCCTTTCT-3′ and 5′-TCGAGACCATCCGGCTAAAAAC-3′) to avoid mistyping ID as DD. With these primers, the I allele produced a 290-bp product and the D allele did not amplify; therefore the DD genotype produced no product.

Statistical analysis

Data are expressed as means ± SD. Values for clinical parameters were compared using one-way analysis of variance with Scheffé's *F* test. Stepwise multiple regression analyses with forward elimination were performed to assess independent influences of variables on a-PWV or carotid stiffness β. First, to examine the effects of risk factors in all subjects on the a-PWV and carotid stiffness β, we used age, sex, BMI, smoking, diabetes, hypertension, dyslipidemia, and gene dose of the ACE I allele (DD = 0, ID = 1, II = 2) as independent variables. Second, the following factors were considered in control subjects: age, sex, BMI, cigarette-years, fasting plasma glucose, HDL cholesterol, non-HDL cholesterol, systolic and diastolic blood pressure, and gene dose of the I allele. Last, the following factors were considered in patients with type 2 diabetes: age, sex, BMI, cigarette-years, duration of diabetes, HbA_{1c}, HDL cholesterol, non-HDL cholesterol, systolic and diastolic blood pressure, and gene dose of the I allele. To assess the effects of the gene dose of the I allele on the a-PWV and carotid stiffness β in patients with type 2 diabetes, we used two separate models for a-PWV and carotid stiffness β. In model 1, gene dose of the I allele was not included as an independent variable for the a-PWV and carotid stiffness β, but in model 2, gene dose of the I allele was included. The *F* value was set as 4.0 at each step. All statistical analyses were carried out with Stat View V on a Macintosh computer. A level of $P < 0.05$ was accepted as statistically significant.

RESULTS

a-PWV and carotid stiffness b

The a-PWV and carotid stiffness β in patients with type 2 diabetes were significantly greater than those in age-matched control subjects in all age groups ($P < 0.05$) (Table 1). The a-PWV and carotid stiffness β were significantly higher in patients with type 2 diabetes and hypertension than in those without hypertension (a-PWV, 10.6 ± 2.7 vs. 9.5 ± 2.9, $P < 0.05$; stiffness β, 17.5 ± 8.1 vs. 13.1 ± 5.9, $P < 0.0001$) (data not shown).

Clinical characteristics

Of the diabetic patients, 27 (19%) had been treated with insulin injection, 74 (54%) had received antidiabetic agents, and 36 (26%) had been treated with diet therapy only. In addition, 83 (61%) had been treated for dyslipidemia and 58 (42%) for hypertension.

There were no differences in age or sex distributions between patients with type 2 diabetes and control subjects (Table 1). Systolic blood pressure, diastolic blood pressure, and LDL cholesterol were significantly higher in patients with type 2 diabetes than in the control subjects (Table 1).

Distribution of ACE I/D genotypes

We observed no significant differences in the distribution of the ACE I/D genotypes between patients with type 2 diabetes and

Table 3—*Risk factors affecting a-PWV and stiffness β of carotid artery in all subjects*

Dependent variable	Independent variable	β	*F* value
a-PWV	Diabetes	0.432	134.686
	Age	0.378	101.034
	Gene dose of I allele	0.158	21.056
	Smoking	0.191	19.503
	Sex	−0.137	10.547
	Hypertension	0.097	6.776
Stiffness β	Age	0.424	104.034
	Diabetes	0.266	40.828
	Hypertension	0.154	12.292
	Gene dose of I allele	0.102	6.582
	BMI	0.093	5.238

Parameters assayed in all subjects included age, sex (female = 0, male = 1), BMI, cigarette-years, diabetes (absent = 0, present = 1), hypertension (absent = 0, present = 1), dyslipidemia (absent = 0, present = 1), and gene dose of the I allele (DD = 0, ID = 1, II = 2). β, standard regression coefficient. For a-PWV, $R^2 = 0.537$ ($P < 0.0001$); for stiffness β, $R^2 = 0.380$ ($P < 0.0001$).

control subjects (Table 2). The distributions of the ACE genotypes observed were in agreement with the Hardy-Weinberg proportion and were consistent with other published reports in Japanese subjects (32). In both patients with type 2 diabetes and control subjects, no significant differences among the genotypes of the ACE gene were found with respect to clinical characteristics (Table 2).

Correlation of a-PWV and carotid stiffness β with ACE genotypes

Both the a-PWV and the carotid stiffness β were significantly higher in the patients with the II genotype than in the other ACE genotypes (Table 2). In control subjects, there were no significant differences in a-PWV or carotid stiffness β among the ACE genotypes (Table 2).

Risk factors for increased a-PWV and carotid stiffness β

Multiple regression analysis demonstrated that, in all subjects, risk factors for increased a-PWV were age, the presence of diabetes, gene dose of the ACE I allele, smoking, sex (female), and hypertension (Table 3). The risk factors for increased carotid stiffness β in all subjects were age, the presence of diabetes, hypertension, BMI, and gene dose of the ACE I allele (Table 3).

Multiple regression analysis was performed separately in diabetic and control subjects to assess the risks associated with increased a-PWV and carotid stiffness β. In assessing the effects of ACE genotype on the arterial distensibility (a-PWV and carotid stiffness β) in patients with type 2 diabetes, we considered two models in multiple regression analysis. In the control subjects, age and decreased HDL cholesterol were associated with increased a-PWV (Table 4); age, systolic and diastolic blood pressure, and BMI were associated with increased carotid stiffness β (Table 4). In the diabetic patients, age, sex (female), systolic and diastolic blood pressure, and cigarette smoking were independently associated with increased a-PWV in model 1. When considering the effects of the ACE gene in model 2, gene dose of the ACE I allele was demonstrated as a risk factor for a-PWV (Table 5). Moreover, the risk factors associated with increased carotid stiffness β were age and systolic blood pressure in model 1; gene dose of the ACE I allele was also shown to be a significant risk factor in model 2 (Table 6).

CONCLUSIONS — The results of this study suggest that the I allele of the ACE gene may be associated with stiffening of the large arteries, such as the carotid arteries and thoraco-abdominal aorta, in patients with type 2 diabetes and may therefore be an independent genetic risk factor for cardiovascular disease in patients with type 2 diabetes. The negative relationships observed in this study between a-PWV and diastolic blood pressure in diabetic patients, and between carotid stiffness β and diastolic blood pressure in control subjects, suggest that increased aortic distensibility can decrease diastolic pressure.

We performed multiple regression analysis among all subjects to determine the impact of diabetes, hypertension, and hyperlipidemia on arterial stiffness. The presence of diabetes and hypertension were common major determinants of increases in a-PWV and carotid stiffness β in all subjects. Both hypertension and diabetes are known to impair arterial distensibility (33) and to be major risk factors for cardiovascular diseases in patients with type 2 diabetes.

Multiple regression analysis of data from control subjects revealed that age contributed independently to increases in a-PWV and carotid stiffness β. Arterial stiffness has been demonstrated to increase with age in healthy subjects (34). Our study showed that gene dose of the I allele was not a risk factor for a-PWV or carotid stiffness β in control subjects.

In patients with type 2 diabetes, stepwise multiple regression analysis revealed that age and systolic blood pressure contributed independently to increases in both a-PWV and carotid stiffness β, whereas smoking, sex, and diastolic pressure contributed to a-PWV in model 1; these variables accounted for 50.5% of the variation in a-PWV. In model 2, gene dose of the ACE I allele was demonstrated to be a risk factor for a-PWV. These variables accounted for

Table 4—*Risk factors affecting a-PWV and stiffness β of carotid artery in control subjects*

Dependent variable	Independent variable	β	*F* value
a-PWV	Age	0.480	77.916
	HDL cholesterol	−0.155	8.126
Stiffness β	Age	0.424	157.736
	Systolic blood pressure	0.347	26.954
	Diastolic blood pressure	−0.154	13.987
	BMI	0.102	4.295

Parameters assayed in control subjects included age, sex (female = 0, male = 1), BMI, cigarette-years, fasting plasma glucose, non-HDL and HDL cholesterol levels, systolic and diastolic blood pressure, and gene dose of the I allele (DD = 0, ID = 1, II = 2). β, standard regression coefficient. For a-PWV, $R^2 = 0.244$ ($P < 0.0001$); for stiffness β, $R^2 = 0.454$ ($P < 0.0001$).

Table 5—*Risk factors affecting a-PWV in patients with type 2 diabetes*

Dependent variable	Independent variable	β	*F* value
Model 1			
a-PWV	Age	0.481	104.034
	Diastolic blood pressure	−0.301	13.987
	Sex	−0.273	13.055
	Systolic blood pressure	0.254	9.964
	Smoking	0.243	9.746
Model 2			
a-PWV	Age	0.556	78.052
	Gene dose of I allele	0.311	27.779
	Diastolic blood pressure	−0.282	15.324
	BMI	0.263	12.049

Parameters assayed in diabetic patients included age, sex (female = 0, male = 1), BMI, cigarette-years, fasting plasma glucose, non-HDL and HDL cholesterol levels, systolic and diastolic blood pressure, and gene dose of the I allele (DD = 0, ID = 1, II = 2). β, standard regression coefficient. For model 1, $R^2 = 0.505$ ($P < 0.0001$); for model 2, $R^2 = 0.545$ ($P < 0.0001$).

54.5% of the variation in a-PWV. Similarly, the strongest predictors of carotid stiffness β were age and systolic blood pressure, accounting for 23.2% of the variation in carotid stiffness β in model 1. In model 2, gene dose of the I allele accounted for 31.4% of the variation. Moreover, we observed increased stiffness of the aorta and the carotid artery in type 2 diabetic patients with the II genotype, compared with the ID and DD genotypes. No differences were observed in systolic or diastolic blood pressure or metabolic controls such as serum cholesterol, triglyceride, serum HDL cholesterol, or fasting plasma glucose among the three ACE genotypes in the patients with type 2 diabetes. Our results suggest that gene dose of the ACE I allele might determine the values of the a-PWV and carotid stiffness β in the diabetic state.

ACE activity was not determined in this study, but previous investigations have shown that plasma and tissue ACE activity are increased in proportion to the number of D alleles (35). In fact, increased IMT of the carotid artery has been shown to be associated with higher plasma ACE levels (36) and the presence of the ACE D allele (37). These apparently conflicting results in patients with type 2 diabetes suggest that the mechanisms involved in the development of arterial hypertrophy and stiffness are different. Indeed, it was shown recently that wall thickening of large arteries is not necessarily associated with increased stiffness, indicating that other structural changes occur to regulate arterial elastic properties (38,39). Moreover, chronically low plasma and tissue ACE levels could be responsible for upregulation of ACE receptor expression, and the effect of ACE genotype may occur via mechanisms other than its effect on serum ACE level.

Benetos et al. (40) reported that aortic stiffness was similar among the three ACE I/D genotypes in normotensive subjects, whereas aortic stiffness was slightly but not significantly higher in hypertensive subjects with the II genotype than in those with DD and ID genotypes. Moreover, they showed by multiple regression analysis that the ACE I allele was a risk factor for increased aortic stiffness in hypertensive subjects. Although the subjects in that and the present study were different, the results were consistent. It has been established that hypertension and diabetes are associated with insulin resistance (41,42). The association between the I allele and arterial distensibility in patients with hypertension and diabetes might be associated with the insulin resistance conferred by the ACE I allele. Recently, an association between ACE polymorphism and plasma insulin has been reported in adults (43–45), with increased plasma insulin levels being observed in the presence of the ACE I allele. These results suggest that ACE gene polymorphism might be a genetic modulator of the effects of insulin sensitivity. The links between the renin-angiotensin system and insulin sensitivity are not fully understood, but recent evidence suggests a tight connection with the insulin signaling system (46). In healthy, normotensive subjects, doses of angiotensin II that affect blood pressure increase insulin sensitivity by a hemodynamic mechanism that involves the redistribution of blood flow to skeletal muscles (47). Suppressor doses of angiotensin II have also been shown to increase insulin sensitivity in type 2 diabetic patients (48), suggesting the intervention of nonhemodynamic mechanisms. It is thus conceivable that the slightly reduced ACE expression in ACE I allele carriers is associated with reduced insulin sensitivity.

There have been several reports concerning the association between insulin resistance and arterial distensibility (16,22). Insulin has been reported to have both atherogenic (49) and anti-atherogenic (50) effects in the vasculature. However, the ability of insulin to induce vasodilation is decreased in insulin resistance, which could be due to inactivation of nitric oxide or to a general impairment in the ability of the endothelial nitric oxide production (51). In addition, some of the atherogenic actions of insulin, mediated by the insulin receptor or IGF-1 receptor, may not be susceptible to insulin resistance and may be increased by hyperinsulinemia accompanied by insulin resistance (52). Thus,

Table 6—*Risk factors affecting stiffness β of carotid artery in patients with type 2 diabetes*

Dependent variable	Independent variable	β	*F* value
Model 1			
Stiffness β	Age	0.330	16.708
	Systolic blood pressure	0.254	9.881
Model 2			
Stiffness β	Age	0.324	17.873
	Gene dose of I allele	0.286	15.786
	Systolic blood pressure	0.267	12.090

Parameters assayed in diabetic patients included age, sex (female = 0, male = 1), BMI, cigarette-years, fasting plasma glucose, non-HDL and HDL cholesterol levels, systolic and diastolic blood pressure, and gene dose of the I allele (DD = 0, ID = 1, II = 2). β, standard regression coefficient. For model 1, $R^2 = 0.232$ ($P < 0.0001$); for model 2, $R^2 = 0.314$ ($P < 0.0001$).

insulin at physiologic concentrations and the nondiabetic state may have anti-atherogenic actions that are lost in insulin-resistant states and diabetes. However, we cannot exclude the possibility that the association observed in this study between ACE polymorphism and arterial distensibility may not be directly attributable to functional polymorphism of the ACE gene.

In conclusion, our results suggest that the I allele of the ACE gene might determine the changes in vascular sclerosis in diabetic or hypertensive states. We cannot draw definitive conclusions regarding the causes of increased stiffness in the presence of the ACE I allele based on the present results, and further studies are therefore required.

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