The 807T Allele in α2 Integrin Is Protective Against Atherosclerotic Arterial Wall Thickening and the Occurrence of Plaque in Patients With Type 2 Diabetes

Takaaki Maeno,[1] Hidenori Koyama,[1] Hideki Tahara,[1] Miyoko Komatsu,[1] Masanori Emoto,[1] Tetsuo Shoji,[1] Masaaki Inaba,[1] Takami Miki,[2] Yasuhisa Okuno,[3] and Yoshiki Nishizawa[1]

Polymorphism of α2 integrin (C807T) is shown to be associated with an increased incidence of thrombotic cardiovascular events. However, it is not clear whether this polymorphism is associated with atherosclerotic arterial wall thickening. In this study, we examined the association of C807T polymorphism with arterial wall thickness in 265 control subjects and 272 patients with type 2 diabetes. In all subjects, intima-media thickness of the right carotid artery in the 807TT group (0.649 ± 0.028 mm [SE]) was significantly (P = 0.0228, Scheffe's F test) less than in the 807CC group (0.767 ± 0.033). This effect of polymorphism is gene dose dependent (P = 0.0227, ANOVA). The similar association was also observed in patients with diabetes but not in control subjects. Multiple regression analysis in all subjects revealed that the T allele was inversely (β = −0.095, P = 0.021) associated with intima-media thickness independent of age, HbA_{1c}, and HDL cholesterol. Finally, an inverse relation between the occurrence of carotid plaque and the T allele was observed in patients with diabetes with an adjusted odds ratio of 0.487 (P = 0.031) in multiple logistic regression analyses. These results suggest that the number of 807T alleles in α2 integrin is protective against atherosclerotic arterial wall thickening and the occurrence of plaque in patients with type 2 diabetes. *Diabetes* 51:1523–1528, 2002

Progression of atherosclerosis is known to be related to vascular risk factors such as hypertension, smoking, diabetes, and high cholesterol levels. In addition to these conventional risk factors, genetic predispositions may enhance the risk of cardiovascular events (reviewed in Murata et al. [1]). Initially reported was the relation between a polymorphism of ACE and coronary arterial diseases (2). Other possible genetic risk factors include angiotensin II type I receptor, endothelial nitric oxide synthase, apolipoprotein E, lipoprotein lipase, paraoxonase, methylenetetrahydrofolate reductase, fibrinogen, plasminogen activator inhibitor-1, thrombomodulin, and platelet glycoproteins (GPs; see review [1]).

The platelet membrane α2β1 integrin, also known as GP Ia-IIa, serves as a platelet receptor for collagen (3). It mediates platelet primary adhesion to subendothelial tissues and activation (4,5). The gene encoding α2 integrin has at least eight polymorphisms, including two silent polymorphisms located within the intron (6). C807T (224Phe) and G873A (246Thr) were reported to be in linkage and associated with the expression density of GP Ia-IIa on the platelet surface (7). T807/A873 is associated with a higher expression of the receptor, and C807/G873 is associated with a lower expression density. The expression density of α2 integrin was also shown to be associated with the rate of platelet attachment to type I collagen, even under high shear rates (8).

α2β1 integrin is also expressed in vascular endothelium (9–11). In endothelial cells, α2β1 integrin seems to function as a receptor for laminin as well as collagen (12), with which cells may interact with basement membrane. Thus, α2β1 integrin might regulate the integrity of endothelium. It is not known, however, whether changes in the levels of α2β1 integrin are associated with endothelial cell function, the dysfunction of which is known to be the cause of progression of atherosclerosis (13). Relations between these polymorphisms and the prevalence of myocardial infarction or stroke have been reported, and the 807T allele has been shown to be at risk (14–16). 807TT homozygote was also reported to be associated with an increased risk of cardiovascular mortality, particularly in women with risk factors for cardiovascular diseases (17). However, it is unknown whether the increased risk of cardiovascular mortality is attributable to thrombosis or arterial wall changes.

To examine the hypothesis that a genetic variation on α2 integrin is associated with atherosclerotic vascular changes, we analyzed the association between C807T polymorphism and intima-media thickness (IMT) of the carotid artery examined by ultrasound in a Japanese population of healthy subjects and patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study subjects. Informed consent was obtained from all subjects who enrolled in the study. This study was approved by the Ethical Committee in Osaka City University Medical School. Healthy subjects (n = 265; 93 men and 172 women) who participated in a local health check program were enrolled

From the [1]Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan; the [2]Department of Geriatrics and Neurology, Osaka City University Graduate School of Medicine, Osaka, Japan; and the [3]Department of Cardiovascular Medicine, Osaka City University School of Medicine, Osaka, Japan.

Address correspondence and reprint requests to Hidenori Koyama, Department of Metabolism, Endocrinology and Molecular Medicine (Second Department of Internal Medicine), Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan. E-mail: hidekoyama@med.osaka-cu.ac.jp.

Received for publication 21 October 2001 and accepted in revised form 18 January 2002.

GP, glycoprotein; IMT, intima-media thickness.

TABLE 1
Clinical characteristics of healthy subjects and patients with type 2 diabetes

	Control subjects	Patients with diabetes
n	265	272
Age (years)	54.0 ± 10.0	55.6 ± 12.6
Sex (M/F)	93/172	162/110#
Diabetes duration (years)	—	11.0 ± 8.9
BMI (kg/m^2)	22.9 ± 3.4	23.6 ± 4.5*
SBP (mmHg)	127.5 ± 18.5	129.6 ± 22.0
DBP (mmHg)	78.5 ± 12.7	73.9 ± 11.9*
MBP (mmHg)	94.8 ± 13.7	92.4 ± 13.5*
Smoking index (cigarette-years)	155 ± 307	463 ± 581*
FPG (mmol/l)	5.3 ± 0.69	8.4 ± 2.6*
HbA_{1c} (%)	4.9 ± 0.42	8.9 ± 2.1*
Non–HDL cholesterol (mmol/l)	3.65 ± 0.88	3.96 ± 1.16*
HDL cholesterol (mmol/l)	1.68 ± 0.46	1.26 ± 0.36*
Hypertension	41 (15.5)	123 (45.7)#
Hyperlipidemia	24 (9.1)	87 (32.3)#
Treatments for diabetes		
Sulfonylurea	—	129 (47.4)
α-Glucosidase inhibitor	—	78 (28.6)
Insulin	—	72 (26.4)

Data are means ± SD and *n* (%). *$P < 0.05$ versus control. Student's *t* test; #$P < 0.05$ versus control, χ^2 test. SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; FPG, fasting plasma glucose.

in the study. The mean age was 54.0 ± 10.0 years (mean ± SD). They had no clinical or laboratory evidence of either a history of vascular disorders or any form of diabetes. Patients with type 2 diabetes were 272 unrelated Japanese subjects (162 men and 110 women). Patients had a diagnosis of type 2 diabetes, as defined by World Health Organization criteria (18) and were participating in the diabetes education program at the Diabetes Center in Osaka City University Hospital (Osaka, Japan). The mean age of the patients was 55.6 ± 12.6 years (mean ± SD). Clinical findings were collected from medical records and are summarized in Table 1. Hypertension was defined as a blood pressure higher than 140/90 mmHg or the use of known agents for the treatment of hypertension. Hyperlipidemia was defined as a total cholesterol >5.2 mmol/l, LDL cholesterol >3.4 mmol/l or the use of any drugs for the treatment of hyperlipidemia (19).

DNA extraction and genotyping of C807T polymorphism. Genomic DNA was isolated from peripheral leukocytes according to standard procedures. The GP Ia gene polymorphisms were identified according to a modified protocol previously described (20). In brief, genomic DNA was amplified by PCR using a GeneAmp PCR System 9,700 (Perkin-Elmer). The primers used in the PCR reaction were as follows: (F) 5′ GATTTAACTTTCCCGACTGCCTTC 3′ and (R) 5′ CATAGGTTTTTGGGGAACAGGTGG 3′. Fifty nanograms of genomic DNA were amplified in a 20-μl volume using a reaction mixture containing 1.5 mmol/l $MgCl_2$, 50 mmol/l KCl, 20 mmol/l Tris-HCl (pH 8.4), 200 μmol/l of each deoxynucleotide triphosphate, 0.01 mg/ml BSA, 20 pmol of each PCR primer, and 1 unit of AmpliTaq Gold DNA polymerase (Perkin-Elmer). After initial denaturation at 94°C for 10 min, 35 cycles of amplification were performed (94°C for 1 min, 65°C for 1 min, and 72°C for 2 min). For typing of 807T and 807C polymorphisms, the PCR products (581 bp) were digested with the restriction enzyme *Bgl*II (New England BioLab). Briefly, 1 μl *Bgl*II enzyme was added directly to each PCR tube. The PCR products were digested at 37°C for at least 2 h and then separated on a 2% agarose gel in 1× Tris acetate EDTA buffer. The gel was stained with ethidium bromide and visualized under ultraviolet light.

Ultrasonography. Ultrasonographic scanning of the carotid artery was performed by high-resolution real-time ultrasonography with a 10-MHz in-line Sectascanner (SSD 650 CL, Aloka) as described previously (21–23). Each subject was examined in the supine position. The examination included the carotid bulb and 4 cm of the right common carotid artery. The site of the most advanced atherosclerotic lesion was examined in the longitudinal and transverse projections to record the maximum IMT (24). IMT was defined as the distance between the leading edges of the lumen-intima interface and the media-adventitia interface of the far wall. The scan converter (Nexus) provided a wide dynamic range and a pixel size of 0.047 mm. The coefficient of variation for carotid artery-IMT was 3.6% (21). The occurrence of carotid plaques was determined as described previously (25).

Biochemical analyses. Plasma glucose levels were measured by the glucose oxidation method, HbA_{1c} by high-pressure liquid chromatography (normal range 4.0–5.5%), and plasma insulin levels by immunoradiometric assay (Insulin RIA bead II kit; Dainabot, Tokyo, Japan). Serum levels of creatinine, total cholesterol, and HDL cholesterol were measured by enzymatic methods adapted to an autoanalyzer (Hitachi 7470; Hitachi, Tokyo, Japan). Non–HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol.

Statistical analyses. Statistical analyses were performed with the StatView V software (SAS Institute, Cary, NC). Student's *t* test, χ^2 test, or ANOVA combined with multiple comparison tests (Scheffe's type) were performed for comparisons of the groups. Simple or multiple regression analyses were performed to evaluate the relation among factors. Predictive variables for the occurrence of carotid plaques were analyzed by logistic regression analysis. $P < 0.05$ was considered significant.

RESULTS

To explore the clinical significance of the α2 integrin C807T polymorphism, we performed a cross-sectional study among 265 nondiabetic healthy subjects and 272 patients with type 2 diabetes. The clinical characteristics of the subjects are shown in Table 1. There was no significant difference in age between healthy subjects and patients with diabetes. The smoking index was significantly higher in patients with diabetes. The number of subjects with hypertension was significantly higher in patients with diabetes, although there was no significant difference in systolic blood pressure between the groups, possibly because of the successful medical treatment. The number of subjects with hyperlipidemia was also significantly higher in patients with diabetes. Non–HDL cholesterol levels were significantly higher and HDL cholesterol levels were significantly lower in patients with diabetes than in healthy subjects.

The numbers of subjects for 807CC, 807CT, and 807TT were 86 (32.5%), 122 (46.0%), and 57 (21.5%), respectively, in the healthy group and 94 (32.5%), 131 (48.2%), and 47 (17.3%) in patients with diabetes, respectively; the distribution between the groups was not significantly different in a χ^2 test ($P = 0.461$).

Clinical parameters of total subjects in relation to the C807T polymorphism are shown in Table 2. No significant difference was observed among three C807T genotypes with regard to age, smoking index, BMI, blood pressure, fasting plasma glucose, HbA_{1c}, and serum levels of non–HDL cholesterol and HDL cholesterol. The distribution of sex and subjects with hypertension or hyperlipidemia were also not significantly different among the groups.

Figure 1*A* shows the comparison of IMT of the right carotid artery among the groups with the 807CC, 807CT, and 807TT genotypes. In all subjects, the gene dose-dependent suppressive effect of the 807T allele on the IMT was observed. The IMT of the 807TT group was significantly lower than that of the 807CC group. A similar dose-dependent suppressive effect of the 807T allele was also observed in patients with diabetes, with the IMT of the 807TT group significantly lower than that of the 807CC group. No significant suppressive effects were observed in healthy subjects. Occurrence of plaque in the right carotid artery among three groups is shown in Fig. 1*B*. In patients with diabetes, occurrence of plaque is significantly more frequent in those who have the 807C allele than in those who have the 807T allele in a χ^2 test.

Table 3 shows the results of simple regression analyses

TABLE 2
Clinical characteristics in relation to C807T polymorphism (N = 537)

	807CC	807CT	807TT	P
n	180	253	104	
Age (years)	54.8 ± 12.4	55.3 ± 11.3	53.7 ± 9.9	NS
Sex (M/F)	80/100	132/121	45/59	NS
Smoking index (cigarette- years)	294 ± 479	336 ± 525	263 ± 403	NS
BMI (kg/m^2)	23.4 ± 4.1	23.1 ± 3.9	23.5 ± 4.3	NS
SBP (mmHg)	128.1 ± 20.4	130.0 ± 20.3	125.7 ± 20.4	NS
DBP (mmHg)	75.9 ± 12.5	76.5 ± 12.5	76.0 ± 12.6	NS
MBP (mmHg)	93.3 ± 13.5	94.3 ± 13.6	92.6 ± 13.7	NS
FPG (mmol/l)	6.9 ± 2.5	6.9 ± 2.4	6.8 ± 2.5	NS
HbA_{1c} (%)	7.1 ± 2.6	6.9 ± 2.4	6.8 ± 2.8	NS
Non–HDL cholesterol (mmol/l)	3.79 ± 1.10	3.83 ± 1.04	3.79 ± 0.93	NS
HDL cholesterol (mmol/l)	1.48 ± 0.46	1.45 ± 0.47	1.51 ± 0.47	NS
Diabetes	94 (52.2)	131 (51.8)	47 (45.2)	NS
Hypertension	53 (29.8)	88 (34.9)	23 (22.1)	NS
Hyperlipidemia	36 (20.2)	60 (23.8)	15 (14.4)	NS

Data are mean ± SD and n (%). ANOVA was done for the comparison among three groups of parameters except distribution of sex, hypertension, and hyperlipidemia. Distribution of sex, diabetes, hypertension, and hyperlipidemia among three groups was analyzed by χ^2 test. SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; FPG, fasting plasma glucose.

of factors correlating IMT. In addition to its inverse relation with the numbers of the 807T allele, carotid IMT was significantly positively correlated with age, smoking index, systolic blood pressure, fasting plasma glucose, and HbA_{1c} and negatively correlated with serum levels of HDL cholesterol in all subjects. In patients with diabetes, only age was significantly positively associated with carotid IMT. IMT in male subjects (0.766 ± 0.023 [SE]) was significantly ($P < 0.0001$, Student's t test) higher than that in the female population (0.679 ± 0.020). This effect of gender on carotid IMT was not observed in patients with diabetes (male, 0.845 ± 0.033 (SE); female, 0.814 ± 0.042, NS). We also compared the IMT values between groups with or without diabetes, hypertension, or hyperlipidemia. Patients with diabetes (0.833 ± 0.026) showed significantly ($P < 0.0001$, Student's t test) higher IMT values than subjects without diabetes (0.613 ± 0.014). Likewise, IMT in subjects with hypertension (0.829 ± 0.030) was significantly ($P < 0.0001$, Student's t test) higher than those in control subjects (0.671 ± 0.017). The subjects with hyperlipidemia tended to have higher IMT values (0.774 ± 0.034) than control subjects (0.705 ± 0.0171; $P = 0.067$).

To clarify whether C807T polymorphism is independently associated with IMT levels, we performed multiple regression analysis in all subjects or patients with diabetes (Table 4). In a model that included age, sex, smoking index, systolic blood pressure, HbA_{1c}, non–HDL cholesterol, and HDL cholesterol as variables (model A, $R^2 = 0.196$, $P < 0.0001$), the number of 807T alleles was demonstrated to be a significant independent variable together with age, HbA_{1c}, and serum levels of HDL cholesterol in all subjects. In patients with diabetes, the number of 807T alleles was a borderline significant independent variable together with age. In another model that included age, sex, smoking index, and presence of diabetes, hypertension, or hyperlipidemia (model B, $R^2 = 0.201$, $P < 0.0001$), the number of 807T alleles was also a significant independent variable together with age and the presence of diabetes. Even restricted to patients with diabetes, the number of 807T allele was also a significant independent variable along with age in this model except for the presence of diabetes.

Finally, we performed multiple logistic regression analyses of factors associated with the presence or absence of carotid plaque in all subjects (Table 5). In model A with age, sex, smoking index, systolic blood pressure, HbA_{1c}, non–HDL cholesterol, HDL cholesterol ,and the number of T alleles as independent variables, an inverse relation between the occurrence of carotid plaque and T alleles was observed with an adjusted odds ratio (OR) of 0.494 (95% CI, 0.267–0.916; $P = 0.025$). Model B (age, sex, smoking index, presence of diabetes, hypertension, or hyperlipidemia and T alleles as independent variables) also had an adjusted OR of 0.486 (95% CI, 0.262–0.902; $P = 0.022$) for the inverse relation between carotid plaque and T alleles, suggesting that the 807TT polymorphism is an independent protective factor against carotid plaque. A similar relation between carotid plaque and T allele numbers was also observed in patients with diabetes.

DISCUSSION

We analyzed the C807T polymorphism of α2 integrin in 265 healthy subjects and 272 Japanese patients with type 2 diabetes. In accordance with previous findings (26), we found an equal distribution of this polymorphism in both groups, suggesting that this polymorphism may not be associated with the onset and/or progression of type 2 diabetes. However, the C807T polymorphism was significantly associated with carotid IMT and carotid plaque, so this genotype may be related to arterial wall thickening. The 807T allele seems to be protective against atherosclerosis, and this effect is highlighted in individuals with type 2 diabetes, not in healthy subjects.

Several gene polymorphisms have been shown to be associated with levels of carotid IMT in patients with type 2 diabetes. Both methylenetetrahydrofolate reductase (C677T) polymorphism and paraoxonase (Gln192Arg) polymorphism have been reported to be positively associated with carotid IMT (27). However, some recent studies reported no association (28,29). An insertion/deletion

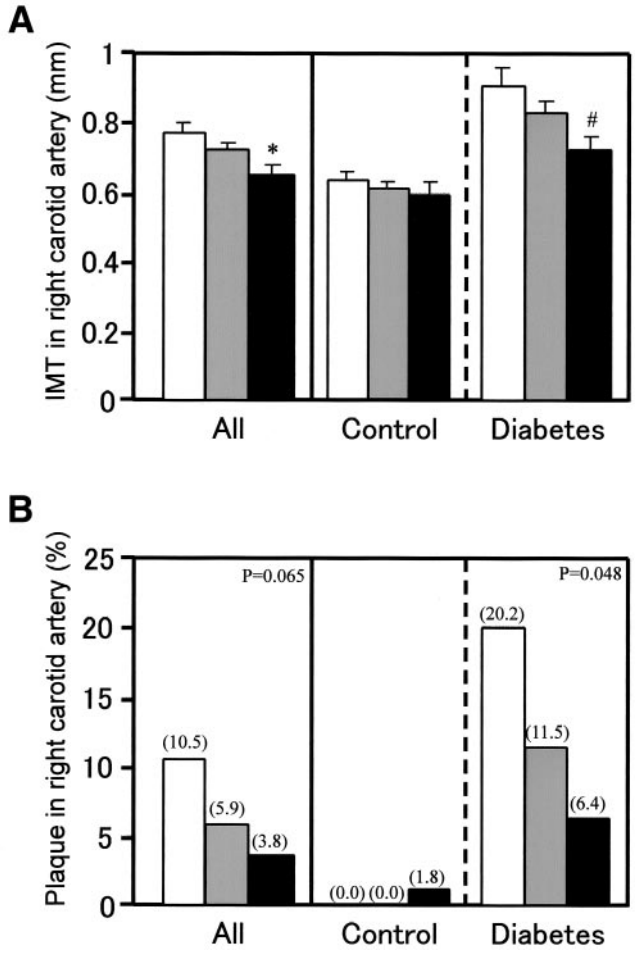

FIG. 1. *A*: Effects of C807T polymorphism of α2 integrin on IMT in the right carotid artery. Right carotid IMT was determined by ultrasound in 265 healthy subjects (control) and in 272 patients with type 2 diabetes. Open column, 807CC; gray column, 807CT; closed column, 807TT. The suppressive effect of the T allele was gene dose-dependently observed in all subjects and in patients with type 2 diabetes ($P < 0.05$, ANOVA). *#$P < 0.05$ versus 807CC, multiple comparison (Scheffe's type). *B*: Occurrence of plaque in the right carotid artery among groups with 807CC, 807CT, and 807TT genotypes. The effect of the allele on plaque formation was statistically analyzed by a χ^2 test. Open column, 807CC; gray column, 807CT; closed column, 807TT.

polymorphism in intron 16 of the ACE gene was previously reported as a risk factor for myocardial infarction (2), and Kogawa et al. (23) showed that the maximum carotid IMT in patients with type 2 diabetes with the D positive genotype (ID + DD) was greater than that in subjects with the II genotype. Associations between other candidate polymorphisms and carotid IMT in patients with type 2 diabetes were also recently reported. Leucine 7 to proline 7 polymorphism in the neuropeptide Y gene was associated with advanced carotid atherosclerosis (30), and mitochondrial genotype Mt5178A was shown to be associated with lower carotid IMT values compared with the MT5178C allele (31). We showed in this study that the C807T polymorphism of α2 integrin is another candidate polymorphism involved in carotid IMT and carotid plaque.

α2 Integrin, forming a heterodimer complex with β1 integrin, is one of the major collagen receptors expressed in a variety of cells including platelets and vascular cells (3,9,10). On the platelet surface, the 807TT genotype was reported to be associated with a higher expression of the receptor and 807CC with a lower expression density. Recently, the 807TT genotype was reported to be a risk factor for the prevalence of myocardial infarction or stroke (14–16). These findings suggest that higher numbers of collagen receptor on the platelet surface is associated with a higher risk of thrombotic events. It is not known, however, whether the association between this polymorphism and cardiovascular events is due to differences in thrombosis or changes in arterial wall thickness. To address this question, we evaluated the association of the C807T polymorphism with carotid IMT and carotid plaque, clinical markers of early atherosclerosis.

TABLE 3
Simple regression analyses of the associations between the carotid IMT and clinical parameters

	All subjects (n = 537)		Patients with diabetes (n = 272)	
	r	P	r	P
Age	0.310	<0.0001	0.344	<0.0001
Smoking index	0.202	<0.0001	0.104	0.109
BMI	0.016	0.727	−0.041	0.518
SBP	0.110	0.014	0.057	0.377
DBP	−0.037	0.402	−0.078	0.223
MBP	0.031	0.479	−0.015	0.817
FPG	0.176	<0.0001	−0.077	0.226
HbA_{1c}	0.221	<0.0001	−0.070	0.271
Non–HDL cholesterol	0.129	0.0037	0.020	0.758
HDL cholesterol	−0.210	<0.0001	−0.012	0.854

SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; FPG, fasting plasma glucose.

In contrast to the relation between the 807TT genotype with a higher risk of cardiovascular events and mortality (14–17), the present findings clearly show the protective effects of the 807T allele of α2 integrin on atherosclerotic arterial wall thickening. First, the IMT levels of 807TT subjects were significantly lower than those of 807CC subjects. The suppressive effects of the 807T allele was dose dependent. The suppressive effect of the 807T allele on carotid IMT was also observed when the subjects were restricted to a population with diabetes. Multiple regression analyses showed that the suppressive effect of the 807T allele on carotid IMT was independent of the clinical parameters of diabetes and hyperlipidemia. Second, the inverse relation between the occurrence of carotid plaque and T allele was observed with an adjusted OR of 0.494 in multiple logistic regression analysis. Again, this effect of the allele was independent of clinical parameters of diabetes. Thus, collagen receptors may be protective against atherosclerotic arterial wall thickening, although they seem to be prothrombotic on the platelet surface.

Collagen receptors in endothelial cells may be important in the maintenance of the endothelium via attachment to the basement membrane and adhesive GPs (10). Thus, it is important to know whether C807T polymorphism of α2 integrin is linked to altered integrin expression in endothelial cells. Provided this is the case as on the platelet surface, the lower numbers of collagen receptors may be associated with the loose integrity and

TABLE 4
Multiple regression analyses of factors that affect carotid IMT

	All subjects		Patients with diabetes	
	β value	*P* value	β value	*P* value
Model A				
Age	0.300	<0.0001	0.357	<0.0001
Sex	−0.015	0.768	−0.020	0.796
Smoking index	0.062	0.231	−0.011	0.890
SBP	0.075	0.073	0.049	0.440
HbA_{1c}	0.137	0.0021	−0.032	0.615
Non–HDL cholesterol	0.045	0.295	0.059	0.350
HDL cholesterol	−0.142	0.0026	−0.069	0.294
T allele numbers	−0.095	0.021	−0.112	0.074
	$R^2 = 0.196$, $P < 0.0001$		$R^2 = 0.150$, $P < 0.0001$	
Model B				
Age	0.286	<0.0001	0.345	<0.0001
Sex	−0.044	0.378	−0.019	0.807
Smoking index	0.040	0.443	0.015	0.850
Presence of diabetes	0.276	<0.0001	—	—
Presence of hypertension	0.031	0.497	−0.006	0.930
Presence of hyperlipidemia	−0.077	0.079	−0.078	0.246
T allele numbers	−0.108	0.0079	−0.128	0.038
	$R^2 = 0.201$, $P < 0.0001$		$R^2 = 0.137$, $P < 0.0001$	

SBP, systolic blood pressure.

dysfunction of the endothelium, which is now regarded as one of the initial events that lead to progression of atherosclerosis (13). There is evidence that endothelium-dependent vasodilatation, one of the markers of endothelial functions, is impaired in patients with diabetes (32–34). Thus, endothelial dysfunction present in patients with diabetes may facilitate the contribution of the α2 integrin genotype on progression of atherosclerosis. It is intriguing to clarify the association between this polymorphism and endothelial dysfunction in a population with diabetes in a future study.

Finally, the suppressive effect of 807TT genotype on atherosclerotic arterial thickening is observed only in patients with diabetes, not in the healthy population. This may be because arterial wall thickness of all genotypes of control subjects is less than that of even the most protected genotype of patients with diabetes (Fig. 1). Without the pathological influence of diabetes or other insult, there may not be much to protect against in the control subjects.

In conclusion, C807T polymorphism is associated with carotid atherosclerosis in patients with type 2 diabetes, with the 807TT genotype protective against it.

TABLE 5
Multiple logistic regression analyses of factors that affect occurrence of carotid plaque

	All subjects	Patients with diabetes
Model A		
Age	1.103 (1.054–1.154)**	1.070 (1.018–1.125)**
Sex (female)	0.548 (0.208–1.445)	0.674 (0.236–1.928)
Smoking index	1.000 (0.999–1.001)	1.000 (0.999–1.001)
Duration of diabetes	—	1.030 (0.987–1.076)
SBP	1.004 (0.986–1.022)	1.002 (0.984–1.021)
HbA_{1c}	1.230 (1.053–1.438)*	0.915 (0.727–1.153)
Non–HDL cholesterol	1.045 (0.726–1.503)	1.091 (0.760–1.564)
HDL cholesterol	0.430 (0.143–1.292)	0.920 (0.266–3.186)
T allele numbers	0.494 (0.267–0.916)*	0.487 (0.253–0.937)*
	$R^2 = 0.205$, $P < 0.0001$	$R^2 = 0.141$, $P = 0.002$
Model B		
Age	1.077 (1.029–1.127)**	1.061 (1.011–1.113)*
Sex (female)	0.603 (0.230–1.581)	0.622 (0.226–1.712)
Smoking index	1.000 (0.999–1.001)	1.000 (0.999–1.001)
Duration of diabetes		1.039 (0.996–1.084)
Presence of diabetes	23.79 (3.08–184.02)**	—
Presence of hypertension	1.546 (0.673–3.551)	1.432 (0.599–3.421)
Presence of hyperlipidemia	1.648 (0.730–3.724)	1.816 (0.775–4.256)
T allele numbers	0.486 (0.262–0.902)*	0.445 (0.232–0.854)*
	$R^2 = 0.280$, $P < 0.0001$	$R^2 = 0.156$, $P < 0.0001$

Data are OR (95% CI). *$P < 0.05$; **$P < 0.01$. SBP, systolic blood pressure.

ACKNOWLEDGMENTS

This study was supported by a Grant-in-Aid for scientific research (13671197 to H.K.) from the Ministry of Education, Science and Culture, Japan. We thank T. Yamada, Osaka City University, for DNA extraction.

REFERENCES

1. Murata M, Kawano K, Matsubara Y, Ishikawa K, Watanabe K, Ikeda Y: Genetic polymorphisms and risk of coronary artery disease. *Semin Thromb Hemost* 24:245–250, 1998
2. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, et al: Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 359:641–644, 1992
3. Santoro SA, Zutter MM: The alpha 2 beta 1 integrin: a collagen receptor on platelets and other cells. *Thromb Haemost* 74:813–821, 1995
4. Nieuwenhuis HK, Akkerman JW, Houdijk WP, Sixma JJ: Human blood platelets showing no response to collagen fail to express surface glycoprotein Ia. *Nature* 318:470–472, 1985
5. Handa M, Watanabe K, Kawai Y, Kamata T, Koyama T, Nagai H, Ikeda Y: Platelet unresponsiveness to collagen: involvement of glycoprotein Ia-IIa (alpha 2 beta 1 integrin) deficiency associated with a myeloproliferative disorder. *Thromb Haemost* 73:521–528, 1995
6. Kamata T, Puzon W, Takada Y: Identification of putative ligand binding sites within I domain of integrin alpha 2 beta 1 (VLA-2, CD49b/CD29). *J Biol Chem* 269:9659–9663, 1994
7. Kunicki TJ, Kritzik M, Annis DS, Nugent DJ: Hereditary variation in platelet integrin alpha 2 beta 1 density is associated with two silent polymorphisms in the alpha 2 gene coding sequence. *Blood* 89:1939–1943, 1997
8. Kritzik M, Savage B, Nugent DJ, Santoso S, Ruggeri ZM, Kunicki TJ: Nucleotide polymorphisms in the α2 gene define multiple alleles that are associated with differences in platelet α2 β1 density. *Blood* 92:2382–2388, 1998
9. Zutter MM, Santoro SA: Widespread histologic distribution of the alpha 2 beta 1 integrin cell-surface collagen receptor. *Am J Pathol* 137:113–120, 1990
10. Luscinskas FW, Lawler J: Integrins as dynamic regulators of vascular function. *FASEB J* 8:929–938, 1994
11. Bahou WF, Potter CL, Mirza H: The VLA-2 (alpha 2 beta 1) I domain functions as a ligand-specific recognition sequence for endothelial cell attachment and spreading: molecular and functional characterization. *Blood* 84:3734–3741, 1994
12. Languino LR, Gehlsen KR, Wayner E, Carter WG, Engvall E, Ruoslahti E: Endothelial cells use alpha 2 beta 1 integrin as a laminin receptor. *J Cell Biol* 109:2455–2462, 1989
13. Ross R: Atherosclerosis: an inflammatory disease. *N Engl J Med* 340:115–126, 1999
14. Carlsson LE, Santoso S, Spitzer C, Kessler C, Greinacher A: The α2 gene coding sequence T807/A873 of the platelet collagen receptor integrin α2β1 might be a genetic risk factor for the development of stroke in younger patients. *Blood* 93:3583–3586, 1999
15. Moshfegh K, Wuillemin WA, Redondo M, Lammle B, Beer JH, Liechti-Gallati S, Meyer BJ: Association of two silent polymorphisms of platelet glycoprotein Ia/IIa receptor with risk of myocardial infarction: a case-control study. *Lancet* 353:351–354, 1999
16. Santoso S, Kunicki TJ, Kroll H, Haberbosch W, Gardemann A: Association of the platelet glycoprotein Ia C807T gene polymorphism with nonfatal myocardial infarction in younger patients. *Blood* 93:2449–2453, 1999
17. Roest M, Banga JD, Grobbee DE, de Groot PG, Sixma JJ, Tempelman MJ, van der Schouw YT: Homozygosity for 807 T polymorphism in α(2) subunit of platelet α(2)β(1) is associated with increased risk of cardiovascular mortality in high-risk women. *Circulation* 102:1645–1650, 2000
18. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. 1. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
19. Association AD: Detection and management of lipid disorders in diabetes (Consensus Statement). *Diabetes Care* 19 (Suppl. 1):S96–S102, 1996
20. Di Paola J, Federici AB, Mannucci PM, Canciani MT, Kritzik M, Kunicki TJ, Nugent D: Low platelet α2β1 levels in type I von Willebrand disease correlate with impaired platelet function in a high shear stress system. *Blood* 93:3578–3582, 1999
21. 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
22. 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
23. Kogawa K, Nishizawa Y, Hosoi M, Kawagishi T, Maekawa K, Shoji T, Okuno Y, Morii H: Effect of polymorphism of apolipoprotein E and angiotensin-converting enzyme genes on arterial wall thickness. *Diabetes* 46:682–687, 1997
24. 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
25. Bonithon-Kopp C, Touboul P-J, Berr C, Leroux C, Mainard F, Courbon D, Ducimetiere P: Relation of intima-media thickness to atherosclerotic plaques in carotid arteries: the Vascular Aging (EVA) Study. 16:310–316, 1996
26. Matsubara Y, Murata M, Maruyama T, Handa M, Yamagata N, Watanabe G, Saruta T, Ikeda Y: Association between diabetic retinopathy and genetic variations in α2β1 integrin, a platelet receptor for collagen. *Blood* 95:1560–1564, 2000
27. Arai K, Yamasaki Y, Kajimoto Y, Watada H, Umayahara Y, Kodama M, Sakamoto K, Hori M: Association of methylenetetrahydrofolate reductase gene polymorphism with carotid arterial wall thickening and myocardial infarction risk in NIDDM. *Diabetes* 46:2102–2104, 1997
28. Cao H, Girard-Globa A, Serusclat A, Bernard S, Bondon P, Picard S, Berthezene F, Moulin P: Lack of association between carotid intima-media thickness and paraoxonase gene polymorphism in non-insulin dependent diabetes mellitus. *Atherosclerosis* 138:361–366, 1998
29. Mazza A, Motti C, Nulli A, Marra G, Gnasso A, Pastore A, Federici G, Cortese C: Lack of association between carotid intima-media thickness and methylenetetrahydrofolate reductase gene polymorphism or serum homocysteine in non-insulin-dependent diabetes mellitus. *Metabolism* 49:718–723, 2000
30. Niskanen L, Karvonen MK, Valve R, Koulu M, Pesonen U, Mercuri M, Rauramaa R, Toyry J, Laakso M, Uusitupa MI: Leucine 7 to proline 7 polymorphism in the neuropeptide Y gene is associated with enhanced carotid atherosclerosis in elderly patients with type 2 diabetes and control subjects. *J Clin Endocrinol Metab* 85:2266–2269, 2000
31. Matsunaga H, Tanaka Y, Tanaka M, Gong JS, Zhang J, Nomiyama T, Ogawa O, Ogihara T, Yamada Y, Yagi K, Kawamori R: Antiatherogenic mitochondrial genotype in patients with type 2 diabetes. *Diabetes Care* 24:500–503, 2001
32. Bucala R, Tracey KJ, Cerami A: Advanced glycosylation products quench nitric oxide and mediate defective endothelium-dependent vasodilatation in experimental diabetes. *J Clin Invest* 87:432–438, 1991
33. Johnstone M, Creager S, Scales K, Cusco J, Lee B, Creager M: Impaired endothelium-dependent vasodilation in patients with insulin- dependent diabetes mellitus. *Circulation* 88:2510–2516, 1993
34. Kawagishi T, Matsuyoshi M, Emoto M, Taniwaki H, Kanda H, Okuno Y, Inaba M, Ishimura E, Nishizawa Y, Morii H: Impaired endothelium-dependent vascular responses of retinal and intrarenal arteries in patients with type 2 diabetes. *Kidney Int* 19:2509–2516, 1999