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The K469E polymorphism of the intercellular adhesion molecule-1 gene is associated with plasma fibrinogen level in type 2 diabetes

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Abstract

Intercellular adhesion molecule-1 (ICAM-1) is involved in inflammation and development of atherosclerotic change of vascular endothelium. The aim of the present study is to investigate whether K469E polymorphism of the ICAM-1 gene is associated with various clinical factors including plasma fibrinogen in patients with type 2 diabetes. ICAM-1 gene polymorphism was examined using polymerase chain reaction and restriction enzyme analysis in 360 type 2 diabetic patients. Plasma fibrinogen levels and other clinical variables were measured as well as circulating soluble ICAM-1 (sICAM-1) levels by enzyme-linked immunosorbent assay. The distribution of ICAM-1 genotypes, EE, EK, and KK, was not significantly different between type 2 diabetes and 152 healthy control subjects. Among 3 groups according to ICAM-1 genotypes in type 2 diabetes, no difference was found in adiposity, glycemic control, lipid profile, insulin sensitivity evaluated by homeostasis model assessment, or sICAM-1. Regarding fibrinogen, the patients with E allele showed significantly lower plasma fibrinogen levels in a dose-dependent manner ($P = .033$). Spearman rank correlation analyses revealed that ICAM-1 genotype showed significant correlation with plasma fibrinogen level ($P < .001$). In multiple regression analysis, ICAM-1 genotype was independent contribution factor of plasma fibrinogen level as well as high-density lipoprotein–cholesterol and urinary albumin excretion ($R^2 = 0.148$, $P < .001$). In conclusion, K469E polymorphism of the ICAM-1 gene had impact on plasma fibrinogen level independently of other clinical factors in 360 type 2 diabetic patients, suggesting that fibrinogen is a candidate which links the ICAM-1 gene polymorphism to atherosclerosis.

1. Introduction

Type 2 diabetic patients are exposed to high morbidity in atherosclerotic diseases, especially in cardiovascular disease, which is a major cause of death in this population. Atherosclerotic change requires inflammatory mechanisms and destabilization of atherosclerotic plaque in the progression [1], where various adhesion molecules and cytokines play important roles. Intercellular adhesion molecule-1 (ICAM-1), a transmembrane glycoprotein belonging to the immunoglobulin gene superfamily [2], is enhanced in its expression on the surface of endothelial cell by inflammatory cytokines, such as tumor necrosis factor-α, and leads to leukocytes adherence and transendothelial migration which is considered to be involved in the development of early atherosclerotic lesion [3]. Indeed, it has been reported that ICAM-1 was highly expressed on the endothelium of plaques in human coronary arterial disease [4]. Recently, K469E polymorphism in exon 6 of the ICAM-1 gene was found, and to date, several studies revealed the correlations between the polymorphism and incidence of various atherosclerotic diseases such as coronary heart disease or myocardial infarction [5], ischemic stroke or vascular dementia [6,7], and peripheral arterial occlusive disease [8]. However, the mechanisms how this gene polymorphism modulates susceptibility to such atherosclerotic diseases remain unclear.

Languino et al first demonstrated that plasma fibrinogen, a glycoprotein playing a key role in blood coagulation and inflammation, acted as a ligand of ICAM-1 [9], and recent reports supported the view that the binding of fibrinogen to ICAM-1 induced bridging between leukocytes to endothe-

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lium [10]. In addition, it was reported that plasma fibrinogen regulated ICAM-1 expression on cell surface [11]. Therefore, we presume fibrinogen to be a candidate for mechanism which links K469E polymorphism of the ICAM-1 gene to atherosclerotic diseases.

In the present study, we examined the association between K469E polymorphism of the ICAM-1 gene and various clinical factors including plasma fibrinogen or early atherosclerotic change, arterial intimal-medial thickness (IMT) assessed by ultrasonography, in 360 type 2 diabetic patients.

2. Subjects and methods

2.1. Subjects

Three hundred sixty type 2 diabetic patients, 187 men and 173 women participating in diabetes education programs, were randomly selected for the present study from among patients attending our diabetes center at Osaka City University Hospital. The diagnosis of type 2 diabetes was based on a previous history of diabetes or on the American Diabetes Association criteria [12]. The patients with any insulin therapy were excluded because fasting plasma insulin level, an essential component for the calculation of insulin resistance index determined by homeostasis model assessment (HOMA IR) as described below, may be affected by insulin therapy. The mean values of age and duration of diabetes of the patients were 55.2 ± 12.1 (SD) years old and 8.0 ± 7.0 years, respectively. The mean body mass index (BMI) of the patients was 24.4 ± 4.5 (SD) kg/m^2. One hundred thirteen patients were treated with sulfonylureas, 19 with α-glucosidase inhibitors, 64 with a combination of sulfonylureas and α-glucosidase inhibitors. Thirty patients were receiving angiotensin-converting enzyme inhibitor. Uremic subjects with serum creatinine levels greater than 132.6 μmol/L and other active medical disease were excluded.

Regarding diabetic microangiopathy, 92 patients were affected with (simple, preproliferative, or proliferative) retinopathy. Sixty-five patients had microalbuminuria, defined as urinary albumin excretion (UAE) of 30 to 300 mg/g creatinine, and 40 with overt proteinuria. Twenty-five patients had had cerebrovascular disease as past illness, 14 with coronary arterial disease, and 13 with peripheral vascular disease. The proportion of these microangiopathy and macroangiopathy did not differ among 3 groups according to genotype.

One hundred fifty-two apparently healthy subjects, 79 men and 73 women, participating in the health check program, were also included for comparison of the distribution of ICAM-1 genotypes as a control group. The mean values of age and BMI of the control subjects were 55.2 ± 11.5 (SD) years old and 24.4 ± 2.6 kg/m^2, respectively, and were not different from those in type 2 diabetic patients.

Informed consent was obtained from all participants in the present study, and the study protocol was approved by the University Hospital Ethics Committee (Approval No 307-309).

2.2. Polymerase chain reaction and genotyping

Genomic DNA of each patient was extracted from peripheral blood using standard methods, and polymerase chain reaction (PCR)–restriction fragment length polymorphism was performed for detecting the ICAM-1 gene. Genomic DNA was amplified using the primer set 5′-AGGATGGCACTTTCCCACT-3′ (sense primer) and 5′-GGCTCACTCACAGAGCACAT-3′ (antisense primer). PCR reaction was performed in a final reaction volume of 20 μL, containing 20 pmol of each primer, 10× PCR buffer, 200 μM of each deoxynucleotide triphosphate, and 1 U of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Cetus, Norwalk, Conn) using a GeneAmp PCR System 9700 (Perkin-Elmer). The amplification consisted of 35 cycles of denaturation (1 minute at 95°C), annealing (1 minute at 63°C), and elongation (2 minute at 72°C). Amplified PCR products were subsequently digested for 3 hours at 60°C with the restriction enzyme *Bst*UI (New England BioLabs, Beverly, Mass). The digested products were then electrophoresed on 3% agarose gel in 1× Tris EDTA buffer followed by ethidium bromide staining and ultraviolet visualization. The EE genotype corresponded with coexistence of 136– and 87–base-pair fragments, EK genotype with 223, 136, and 87 base-pair fragment, and KK genotype with only 223 base-pair fragment.

2.3. Ultrasonographic measurements of intimal-medial thickness of common carotid and femoral artery

Ultrasonographic examinations for atherosclerosis of common carotid and femoral arteries in the supine position with slight hyperextension of the neck were performed using an ultrasonic phase-locked echo-tracking system, which was equipped with a high-resolution real-time 7.5-MHz linear scanner (SSD 610; Aloka, Tokyo, Japan) [13,14]. The scan examination included approximately 4 cm of common carotid artery and carotid bulb for the carotid artery and approximately 4 cm of the femoral artery and the bifurcation between the profound and superficial femoral artery. These regions were scanned bilaterally in the longitudinal and transverse projections. The image was focused on the far wall of the artery. The IMT was measured at the site of the most advanced atherosclerotic lesion including plaques which exhibited the greatest distance between the lumen-intimal interface and the media-adventitia interface of the far wall in both carotid and both femoral arteries. We used the larger of the maximal IMT of bilateral common carotid and femoral arteries (CA-IMT and FA-IMT, respectively).

2.4. Measurements

Percentage of body fat was estimated by bioelectrical impedance analysis using the Body Composition Analyzer (BC-118; Tanita Co, Tokyo, Japan).

Table 1
The distribution of K469E polymorphism in exon 6 of the ICAM-1 gene in type 2 diabetes and control subjects

	Type 2 diabetes	Control	*P*
n	360	152	—
Men/women	173/187	73/79	.995[a]
Age (y)	55.2 ± 12.1	55.2 ± 11.5	.995
BMI (kg/m^2)	24.4 ± 4.4	24.4 ± 2.6	.993
Genotypes	13.9/43.9/42.2	11.8/48.0/40.1	.652[a]
EE/EK/KK (%)			(χ^2 = 0.855)

Values are presented as n, or mean ± SD. *P* values in the right column were determined by analysis of variance or by [a]χ^2 test.

Blood were drawn after an overnight fast. Circulating soluble ICAM-1 levels (sICAM-1) were measured in 9 patients among each genotype group, matched for age, sex, and BMI, by a commercially available enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, Minn). Plasma fibrinogen levels were determined by the thrombin-time method. All other biochemical measurements were performed using standard laboratory methods. HOMA IR was calculated from fasting plasma glucose (FPG) and insulin (FIRI) levels according to the report by Matthews et al [15] with the formula: HOMA IR = FIRI in mU/L × FPG in mg/dL/405. In the present study, we used HOMA IR as a surrogate index for insulin resistance [16].

2.5. Statistical analyses

All values are mean ± SE, unless otherwise indicated. Statistical analysis was performed using the StatView 5 system for Windows. For detecting the differences in the distribution of genotypes between type 2 diabetes and control subjects, we used χ^2 test. Kruskal-Wallis test was used for comparisons of the clinical factors among 3 groups according to genotype. Linear simple or multiple regression analysis was performed for analysis of associations among plasma fibrinogen level and clinical covariates including ICAM-1 gene polymorphism. *P* values < .05 were considered statistically significant.

Table 3
Correlation coefficients by simple linear regression analyses or Spearman rank correlation between plasma fibrinogen level and various clinical factors in 360 type 2 diabetic patients

	r Value	*P*
Sex (men = 0, women = 1)	0.137[a]	.018
Age	0.004	.941
BMI	0.064	.307
Percentage of body fat	0.030	.698
Duration of diabetes	0.115	.070
Smoking index	−0.041	.512
Systolic blood pressure	0.146	.013*
HbA1c	0.100	.089
HDL-cholesterol	−0.193	<.001*
Non–HDL-cholesterol	0.181	.002*
UAE	0.224[a]	.002*
HOMA IR	−0.022	.714
ICAM-1 polymorphism (EK or EE = 0, KK = 1)	0.263[a]	<.001*

[a] ρ value by Spearman rank correlation method.
* *P* < .05.

3. Results

The distribution of K469E polymorphism in exon 6 of the ICAM-1 gene in 360 type 2 diabetes and 152 control subjects is shown in Table 1. The distribution of ICAM-1 genotypes, EE, EK, and KK, was not significantly different

Table 2
Clinical characteristics of 360 type 2 diabetic patients divided in 3 groups according to genotype of the K469E polymorphism of ICAM-1 gene

	EE	EK	KK	*P*
n	50	158	152	—
Men/women	17/33	85/73*	85/67*	.029[a]
Age (y)	55.2 ± 1.5	55.1 ± 1.0	55.3 ± 1.0	.910
BMI (kg/m^2)	24.6 ± 0.6	24.7 ± 0.4	24.0 ± 0.4	.599
Percentage of body fat	32.4 ± 1.8	28.4 ± 1.2	28.2 ± 1.1	.092
Duration of diabetes (y)	7.6 ± 1.1	7.9 ± 0.6	8.3 ± 0.7	.577
Smoking index (cigarette-years)	385 ± 109	457 ± 52	419 ± 50	.415
Blood pressure (mm Hg)				
Systolic	130 ± 4	127 ± 2	128 ± 2	.690
Diastolic	76 ± 2	75 ± 1	73 ± 1	.518
HbA1c (%)	8.3 ± 0.3	8.6 ± 0.2	8.8 ± 0.2	.159
HDL-cholesterol (mmol/L)	1.27 ± 0.05	1.25 ± 0.03	1.22 ± 0.03	.327
Non–HDL-cholesterol (mmol/L)	4.11 ± 0.14	3.95 ± 0.08	3.88 ± 0.09	.368
Fibrinogen (mg/dL)	298 ± 8	307 ± 6	326 ± 8	.033
UAE (mg/g creatinine)	92.1 ± 35.4	86.5 ± 26.7	81.8 ± 17.5	.736
HOMA IR	2.44 ± 0.24	2.47 ± 0.15	2.47 ± 0.16	.992
sICAM-1 (ng/mL, n = 9 per group)	250 ± 11	216 ± 29	214 ± 14	.492
CA-IMT (mm)	0.912 ± 0.039	0.972 ± 0.037	1.012 ± 0.041	.692
FA-IMT (mm)	1.139 ± 0.079	1.391 ± 0.073	1.322 ± 0.068	.303

All values are presented as n, or mean ± SE. *P* values in the right column were determined by Kruskal-Wallis test or by [a]χ^2 test.
HbA1c indicates glycated hemoglobin A1c.
* *P* < .05 versus EE.

Table 4
Multiple regression analysis of ICAM-1 polymorphism determining plasma fibrinogen level in 360 type 2 diabetic patients

	β	P
Sex (men = 0, women = 1)	.098	.184
Systolic blood pressure	.063	.403
HDL-cholesterol	−.226	.003
Non-HDL-cholesterol	.045	.531
Log(UAE)	.205	.006
ICAM-1 polymorphism (EK or EE = 0, KK = 1)	.151	.033
R^2	.138	<.001

R^2 indicates coefficient of determination; log(UAE), log-transformed UAE.

between type 2 diabetes and control subjects. Clinical characteristics of type 2 diabetic patients divided into 3 groups according to genotype of the K469E polymorphism of ICAM-1 gene are shown in Table 2. EE genotype had a significantly higher prevalence of female patients than those in EK or KK genotype ($P = .029$), whereas there were no differences among genotypes in age, BMI, percentage of body fat, and blood pressure. No association was found between genotypes and duration of diabetes or glycemic control. There were also no differences in HDL-cholesterol or non–HDL-cholesterol among genotypes. Three groups were equal in insulin sensitivity evaluated by HOMA IR. There were no significant differences in sICAM-1 among genotypes. In addition, neither CA-IMT nor FA-IMT arteries were affected by the genotype. Regarding fibrinogen, the patients with E allele showed significantly lower plasma fibrinogen levels in a dose-dependent manner ($P = .033$).

Second, we examined the correlation between plasma fibrinogen level and various clinical factors by simple regression analyses or by Spearman rank correlation test in case variables strayed out of normal distribution (Table 3). Systolic blood pressure, non–HDL-cholesterol, and UAE were significantly positively correlated with plasma fibrinogen level ($P = .013$, $P = .002$, and $P < .002$, respectively), whereas HDL-cholesterol showed significantly negative correlation with plasma fibrinogen level ($P < .001$). In ICAM-1 genotype, lack of E allele showed significantly positive correlation with plasma fibrinogen level ($P < .001$).

Finally, we performed multiple regression analysis with plasma fibrinogen level as dependent variable and the clinical factors, which had significant correlation with plasma fibrinogen level in simple linear regression analyses or in Spearman rank correlation test, as independent variables. Here, UAE was entered as log-transformed value because of disproportional distribution. ICAM-1 genotype was independent contribution factor of plasma fibrinogen level as well as HDL-cholesterol and log-transformed UAE ($R^2 = 0.138$, $P < .001$, Table 4).

4. Discussion

In the present study, we demonstrated that E allele in K469E polymorphism of the ICAM-1 gene was associated with lower plasma fibrinogen level in 360 type 2 diabetic patients. Furthermore, our multiple regression analysis showed that K469E polymorphism in ICAM-1 gene was significant contributor to plasma fibrinogen level independently of other clinical factors, HDL-cholesterol, and urinary albumin excretion. To our knowledge, this is the first report referring to the association between the ICAM-1 gene polymorphism and plasma fibrinogen level.

ICAM-1 is an adhesion molecule which induces leukocyte–endothelial cell interactions and consequent migration of leukocytes at inflammation sites; therefore, it is considered to contribute to the development of early atherosclerotic lesion [3]. In fact, it has been reported that ICAM-1 expression on the human endothelium was increased in atherosclerotic lesions, such as coronary arterial plaques [4], and that homozygous null ICAM-1 mice fed a fat diet showed a reduced aortic atherosclerotic lesions [17]. Formerly, K469E polymorphism of the ICAM-1 gene has been reported in connection with the susceptibility to various inflammatory and immune diseases, such as Behçet disease [18], multiple sclerosis [19], or inflammatory bowel disease [20]. Because it has been evident that inflammation is the principal cause of atherosclerosis and that these adhesion molecules have gained much attention for playing an important role in the progression, several reports are accumulating that referred to the associations between the polymorphism and incidence of various atherosclerotic diseases, including ischemic heart disease [5], stroke or vascular dementia [6,7], and peripheral arterial occlusive disease [8]. In most of these reports, some of which include diabetic subjects, regardless of ethnical differences, EE genotype was responsible for increasing risk of atherosclerotic diseases independently of the presence of diabetes. However, the mechanisms by which the ICAM-1 gene polymorphism affects the susceptibility to these diseases remain unknown.

It has also been well known that plasma fibrinogen plays an important role in blood coagulation and inflammation. Several investigations have demonstrated that high plasma fibrinogen level was shown in patients with cardiovascular diseases or diabetic microangiopathy. Recently, it has been demonstrated that plasma fibrinogen acts as a ligand of ICAM-1 [9] and that the binding of fibrinogen to ICAM-1 induces bridging between leukocytes to endothelium [10]. K469E polymorphism of the ICAM-1 induces amino acids change in immunoglobulin-like domain 5 and could alter the structure of binding site to ligands including fibrinogen. Our results, which demonstrated that ICAM-1 gene polymorphism interacted with plasma fibrinogen level, therefore, may suggest that plasma fibrinogen modifies the susceptibility to atherosclerotic diseases through the differences of affinity to ICAM-1 on endothelial cell surface according to K469E polymorphism of the ICAM-1. Furthermore, it may be possible that K469E polymorphism of the ICAM-1 modulates fibrinogen mRNA synthesis in liver, which is known to be up-regulated by inflammatory cytokines [21].

The result of our study, in which E allele of the ICAM-1 genotype was demonstrated to be responsible for reduced plasma fibrinogen level, was not consistent to other previous studies about ICAM-1 gene polymorphism reporting EE genotype as a risk factor of atherosclerotic diseases. In fact, we failed to find any association between ICAM-1 genotype and early atherosclerotic change in carotid or femoral arteries assessed by CA-IMT or FA-IMT, respectively. The inconsistency may be caused by the differences in study population, all of which were type 2 diabetic patients in the present study. In type 2 diabetic patients, ICAM-1 may have partly different meaning in its function in progression of vascular damage from nondiabetic subjects. Kamiuchi et al reported that KK genotype of ICAM-1 gene was associated with increased risk for retinopathy in type 2 diabetic patients [22]. There were numerous reports that showed plasma fibrinogen and other coagulation factors to be responsible to progression of diabetic microangiopathy [23,24]. Furthermore, the latest report by Okada et al using ICAM-1–deficient mice revealed critical importance of ICAM-1 in pathogenesis of diabetic nephropathy [25]. Based on these findings, in diabetic patients, it is possible that the interaction of ICAM-1 and fibrinogen have impact rather on microvascular injury than macrovascular lesions which could be affected by other various factors such as lipid profile, although there was no difference in frequency of microvascular complication in our study population including small number of patients with retinopathy (data not shown).

Previous studies [5-8] reported that the influences of ICAM-1 polymorphism on sICAM-1 were not investigated. In the present study, we also showed that there were no significant differences in sICAM-1 level among genotypes. The Atherosclerosis and Insulin Resistance study revealed that circulating level of sICAM-1 was correlated with atherosclerotic changes in carotid and femoral arteries assessed by intima-medial thickness [26], whereas other studies failed to demonstrate a significant correlation between sICAM-1 and atherosclerotic diseases [27]. In fact, sICAM-1 level does not necessarily reflect the amount or activity of expressed ICAM-1 on endothelial cell surface. In addition, whether sICAM-1 may deteriorate atherosclerosis or work protectively as decoy in apprehension of leukocyte remains unknown. One way or another, ICAM-1 gene polymorphism is unlikely to modulate the susceptibility to atherosclerosis through the increase in sICAM-1.

There are some limitations in our study. First, the disequilibrium of sex was found among ICAM-1 genotypes. Although there were no reports which concerned the association between the genotype and sex prevalence, it may be caused by the differences in the population size among genotypes. Second, it is not also known that plasma fibrinogen level could vary in proportion to the degree of fibrinogen–ICAM-1 interaction. Finally, as the most important issue, no correlation was found between plasma fibrinogen levels and CA-IMT or FA-IMT in our type 2 diabetic population, although recent investigations with large study population reported that plasma fibrinogen levels were correlated with IMT [28,29] or increased risk of ischemic heart disease [30,31]. This inconsistency may be caused by the difference in study population. Type 2 diabetes is known to have high plasma fibrinogen levels in proportion to the degree of hyperglycemia [32]; therefore, fibrinogen may have small impact on progression of atherosclerosis in type 2 diabetes than in healthy subjects. Furthermore, arterial wall thickening and plaque instability which is necessary to evoke cardiovascular event may share partly different process in progression. Causal link between ICAM-1 polymorphism and atherosclerosis through fibrinogen is needed to be further investigated.

In conclusion, K469E polymorphism of the ICAM-1 gene is associated with plasma fibrinogen level independently of other clinical factors in 360 type 2 diabetic patients. It could be a clue which links the ICAM-1 gene polymorphism to atherosclerosis.

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