

# 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.

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## 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- $\alpha$ , 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  $55.2 \pm 12.1$  (SD) years old and  $8.0 \pm 7.0$  years, respectively. The mean body mass index (BMI) of the patients was  $24.4 \pm 4.5$  (SD)  $\text{kg/m}^2$ . One hundred thirteen patients were treated with sulfonylureas, 19 with  $\alpha$ -glucosidase inhibitors, 64 with a combination of sulfonylureas and  $\alpha$ -glucosidase inhibitors. Thirty patients were receiving angiotensin-converting enzyme inhibitor. Uremic subjects with serum creatinine levels greater than  $132.6 \mu\text{mol/L}$  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  $55.2 \pm 11.5$  (SD) years old and  $24.4 \pm 2.6 \text{ kg/m}^2$ , 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  $\mu\text{L}$ , containing 20 pmol of each primer,  $10\times$  PCR buffer, 200  $\mu\text{M}$  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  $95^\circ\text{C}$ ), annealing (1 minute at  $63^\circ\text{C}$ ), and elongation (2 minute at  $72^\circ\text{C}$ ). Amplified PCR products were subsequently digested for 3 hours at  $60^\circ\text{C}$  with the restriction enzyme *Bst*UI (New England BioLabs, Beverly, Mass). The digested products were then electrophoresed on 3% agarose gel in  $1\times$  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).

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