The association between insertion/deletion polymorphism of the angiotensin I converting enzyme (ACE) gene and insulin resistance (IR) was investigated in 64 consecutive patients (F/M: 11/53) with angina pectoris without clinically manifest diabetes mellitus who underwent diagnostic coronary angiography. The observed frequency distribution of ACE genotypes did not deviate from that predicted from the Hardy-Weinberg equilibrium in this group. Patients with the ACE-ID genotype had significantly lower IR, as assessed by an oral glucose tolerance test (OGTT) and by homeostatic model assessment (HOMA), compared to those with the ACE-II genotype, as assessed by a multiple comparison analysis. Patients were divided into two groups with low and high HOMA-IR, and the I allele was seen more frequently in the high HOMA-IR group than in the low HOMA-IR group (0.62 vs 0.47, respectively, by χ² test, P < .05). Logistic regression analysis showed that the odds ratio for insulin resistance in patients with the II genotype, compared to those with the ID and DD genotypes (assuming that the I allele has a recessive effect), was 4.0 (95% confidence interval, 1.2 to 16.5; P = .037), after adjusting for the presence of significant coronary atherosclerosis. In conclusion, the D allele was not associated with higher insulin resistance in patients with angina pectoris; that is, patients with the ID and DD genotypes were associated with a significantly lower risk of insulin resistance, compared to those with the II genotype. Am J Hypertens 1999;12:291–297 © 1999 American Journal of Hypertension, Ltd.

KEY WORDS: Genetics, angiotensin I converting enzyme gene, insulin resistance, insertion/deletion polymorphism.
roration of renal function. However, the mechanism of the association of the D allele with the diseased state is not yet clear, and some reports conflict with those mentioned above.5–8

Insulin resistance is frequently found in patients with CHD,9–11 and treatment with ACE inhibitors has been shown to have ameliorative effects in hyperinsulinemia, to improve insulin sensitivity,12–14 and to reduce various complications after a heart attack. The aim of this study was to examine the relationship between polymorphism of the ACE gene and insulin resistance in patients with angina pectoris without clinically manifest diabetes mellitus who underwent diagnostic coronary angiography.

METHODS

Patients The study population consisted of 64 consecutive patients with angina pectoris without clinically manifest diabetes mellitus who underwent diagnostic coronary angiography for suspected or known coronary atherosclerosis or for other reasons (mostly atypical chest pain) at Fukuoka University Hospital from January to July 1997. Patients with acute myocardial infarction (MI) were excluded from the study, but 21 patients had old MI. Patients with heart failure (Killip class ≥2 after MI), hepatic dysfunction, vascular disease (aortitis treated with prednisolone), familial hypercholesterolemia, thyroid dysfunction, or adrenal dysfunction were excluded from the study. Patients who were being treated for diabetes mellitus (DM) and those with or without symptoms of DM and a fasting glucose >140 mg/dL and glycosylated hemoglobin (HbA1c) >7.0% were considered to have manifest DM, and were excluded from the study. Otherwise, a 75-g oral glucose tolerance test (OGTT) was performed the day of admission to avoid the effects of hospitalization on diet. About 98% of the women were in menopause, but none were receiving hormone replacement therapy. This protocol was approved by the ethics committee of Fukuoka University School of Medicine and written informed consent was obtained from all of the subjects.

Coronary Angiography Coronary arteries were cannulated by the Judkins technique15 with 5F catheters, and recorded on Kodak (Rochester, New York) 35 mm cinefilm at a rate of 30 frames/sec. Normal coronary arteries were defined as those with <25% luminal narrowing, and arterial stenosis that produced >50% luminal narrowing was considered significant. In cases with no significant stenosis by coronary arteriogram, acetylcholine (20, 50, and 100 μg) was injected into the right and left coronary arteries to diagnose spastic angina pectoris. When stenotic coronary arteries were found, the presence of stenosis was determined using a computer-assisted coronary angiography analysis system (Mipron 1; Kontron Co., Tokyo, Japan) after the direct intracoronary injection of isosorbide dinitrate (ISDN) (2.5 mg/5 mL solution).15 One minute after the injection of ISDN through the Judkins catheter given over 20 sec, coronary angiography was performed from several projections during 7 min, as described previously.16 The severity of CHD was assessed by Gensini's score (GS).17 Forty-five patients showed GS > 0, and 19 patients showed GS = 0. Thirteen patients showed typical spasm of the epicardial coronary arteries after intracoronary injection of acetylcholine with ST-segment depression of 0.1 mV and angina attack, whereas 6 patients showed mild coronary vasospasm with or without ST-depression and chest pain, but were clinically diagnosed as vasospastic angina pectoris.

Blood Samples and Measurements Blood samples were collected after an overnight fast to measure plasma glucose, insulin, serum lipid, lipoprotein, and plasma fibrinogen concentrations. All of the subjects performed a 75-g oral glucose tolerance test (75-g OGTT), and blood was collected at 0, 30, 60, and 120 min to determine both plasma glucose and insulin concentrations. Blood glucose was measured by the glucose oxidase method, and the plasma insulin concentration was measured by a standard radioimmunoassay.18 The serum total cholesterol (TC) and triglyceride (TG) levels were measured by enzymatic methods.19,20 High-density lipoprotein cholesterol (HDL-C) was measured by the heparin–calcium precipitation method.21 Plasma fibrinogen levels were measured by the Clauss method.22

Insulin Sensitivity/Resistance and Its Calculations Insulin sensitivity was estimated by the ratio of the areas under the insulin and glucose curves in the 75-g OGTT and a HOMA model23 using fasting-specific insulin and glucose concentrations. The total areas under the plasma glucose and insulin concentration curves from 0 to 120 min were calculated by trapezoid methods and are referred to as the glucose (milligram per deciliter per hour) and insulin responses (michunit per milliliter per hour). Insulin sensitivity estimated from the HOMA model has been reported to correlate well with insulin sensitivity measured using a euglycemic clamp. (r = 0.88, P < .0001).23

DNA Extraction and ACE Gene Polymorphisms Genomic DNA was amplified as previously described using the polymerase chain reaction (PCR) with primers flanking the polymorphic region.1–7 Polymerase chain reaction (PCR) products of 490 and 290 base pairs (bp) were separated on 1.5% agarose gels and visualized by ethidium bromide staining.

Statistical Analysis All of the statistical analyses were performed using the SAS software package (Sta-
RESULTS

Table 1 shows the observed frequencies of the three ACE genotypes, and those predicted based on this sample from the Hardy-Weinberg equilibrium. The observed frequency distribution of ACE genotypes did not deviate from the predicted data.

Insulin resistance was only analyzed in patients without manifest DM. Table 2 shows the clinical characteristics of these patients. ACE-II patients had significantly higher fasting insulin concentrations than ID patients (314 ± 65 μU/mL, respectively, *P* < .05). The ratio of the total area under the insulin curve to total area under the glucose curve in II subjects was significantly higher than that in ID patients (47.8 ± 12 μU/mL, respectively, *P* < .05). The ratio of the total area under the insulin curve to total area under the glucose curve in II subjects was significantly higher than that in ID patients (Figure 1D).

Table 2 shows the results of a logistic regression analysis. Box and whisker plots of the total areas under the glucose and insulin curves, the ratio of the area of insulin to the area of glucose, and HOMA insulin resistance are shown in Figure 1. The three genotype groups show a similar area of glucose (Figure 1A), whereas the area of insulin is greater in II than in ID and DD (*P* < .05) (Figure 1B). The ratio of the total area under the insulin curve to total area under the glucose curve in II subjects was significantly higher than that in ID subjects (Figure 1C). Insulin resistance in II patients, as assessed by the HOMA model, was significantly lower than that in II patients (Figure 1D).

The distributions of ACE gene polymorphism in patients with high and low insulin resistance as assessed by both the insulin-to-glucose ratio and the HOMA model are shown in Table 3. A higher frequency of the I allele was observed in the group with high insulin resistance by the HOMA model, compared to the group with low resistance (0.62 ± 0.47, respectively, *P* < .05) (Table 3).

Table 4 shows the results of a logistic regression analysis of the association between insulin resistance.
and the ACE genotype with (Table 4, lower panel) and without (Table 4, upper panel) adjusting for the presence of a significant coronary atherosclerotic lesion. The unadjusted odds ratio for patients with the II genotype, compared to patients with the ID and DD genotype (assuming that the I allele has a recessive effect) was 4.2 (95% confidence interval, 1.2 to 16.8; \( P = .027 \)). The relative risk of a higher insulin resistance associated with the I allele (assuming an additive effect) was 2.3 (95% confidence interval, 1.0 to 5.6; \( P = .054 \)). The relative risk of a higher insulin resistance associated with the II and ID genotype, compared to patients with the DD genotype (assuming that the I allele has a dominant effect) was 1.6 (95% confidence interval, 0.4 to 6.9; \( P = \text{NS} \)). Similar data were obtained even after adjusting for the presence of a significant coronary atherosclerotic lesion (Table 4, lower panel).

**DISCUSSION**

The patients studied all had clinical symptoms of chest pain without clinically manifest diabetes mellitus. Thirteen patients were diagnosed as typical vasospastic angina pectoris, and 45 patients showed coronary atherosclerosis. Six patients were clinically diagnosed as having vasospastic angina pectoris, but acetylcholine-induced vasospasms were mild. Because acetylcholine-induced vasoconstriction is observed in atherosclerotic segments and endothelial dysfunction produces atherosclerotic lesions,\(^27,28\) we combined all of the patients as having angina pectoris. A statistical analysis was also conducted with and without adjusting for the presence of significant coronary atherosclerosis, as shown in Table 4. The frequency distribution of ACE genotypes in patients was similar to that predicted from the Hardy-Weinberg equilibrium\(^26\); however, in general, patients with several known risk factors, including diabetes mellitus, may be more likely to be referred for coronary angiography. Thus, a selection bias may exist, as the patients studied here essentially differ from healthy controls and patients with CHD.

The association between ACE genotypes and insulin sensitivity was not investigated in normal controls in our study. However, Chiu and MaCarthy\(^29\) showed that the I allele is associated with insulin resistance in glucose tolerant and normotensive men. Katsuya et

**TABLE 3. FREQUENCY DISTRIBUTION OF ACE POLYMORPHISM IN GROUPS WITH HIGH AND LOW SENSITIVITIES TO INSULIN, DEFINED ACCORDING TO THE MEDIAN VALUES OF INSULIN SENSITIVITY**

<table>
<thead>
<tr>
<th>ACE Genotype</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of insulin/area of PG ((\times 10^{-2} \mu\text{U/mg}))</td>
<td>II</td>
</tr>
<tr>
<td>(\geq 31)</td>
<td>11</td>
</tr>
<tr>
<td>Area of insulin/area of PG ((\times 10^{-2} \mu\text{U/mg}))</td>
<td>&lt;31</td>
</tr>
<tr>
<td>Insulin resistance (HOMA) (\geq 3.3)</td>
<td>12</td>
</tr>
<tr>
<td>Insulin resistance (HOMA)</td>
<td>&lt;3.3</td>
</tr>
</tbody>
</table>

PG, plasma glucose.

* Mantel-Haenszel \(\chi^2 = 3.87, P < .05 \).
† \(\chi^2 = 3.15, P = .076 \).
al30 reported that subjects with normal glucose toler-
ance and the ACE-DD genotype had a lower body
mass index, plasma glucose, and insulin response to
OGTT, and that ACE-DD subjects had fewer other risk
factors for CHD. These data suggest that the I allele is
associated with hyperinsulinemia not only in CHD
but also in normal subjects. Although some studies
have shown that the ACE-II genotype is associated
with insulin resistance, other studies have not.6–8 This
discrepancy may depend in part on the patients se-
lected, as such studies were conducted in various
disease states (ie, hypertensives,3 non–insulin-depen-
dent diabetes mellitus,7,30,31 coronary heart disease
patients,6,7 or in different populations7,8,29,32).

In this study, patients with the ACE-II genotype had
significantly higher basal immunoreactive insulin, in-
sulin response to OGTT, insulin resistance, and
plasma fibrinogen concentrations, compared to ID pa-
tients. No significant differences were observed with
regard to other factors among the ACE polymor-
phisms. The odds ratio for insulin resistance for pa-
tients with the II genotype, compared to patients with
the ID and DD genotypes (recessive effect of I allele)
was statistically significant (P = .037). The additive
effect of the I allele for insulin resistance tended to be
significant, whereas the dominant effect of the I allele
was not significant, with or without adjusting for the
presence of significant coronary atherosclerosis. These
results indicate that the D allele was not associated
with insulin resistance; thus, patients with the DD and
ID genotypes were associated with a significantly
lower risk of insulin resistance (Table 4), and the in-
creased cardiovascular risk associated with the DD
genotype does not appear to be mediated through
insulin resistance.

Panahloo et al31 reported that patients with non-
insulin-dependent diabetes mellitus and the DD geno-
type had significantly lower levels of specific insulin
and higher insulin sensitivity than II subjects. In our
recent study of exercise therapy for hypertension, the
ACE I/D polymorphism was associated with a de-
pressor effect of exercise therapy in mild essential
hypertensives33; multiple logistic regression analysis
revealed that the D allele had significant additive,
recessive, and dominant effects on the poor depressor
effect of exercise therapy. The ACE-II genotype may
have a greater variation in the insulin range, as re-
lected in the insulin area, than the DD genotype, and
therefore, may show a better response to either drugs
or exercise.

It has been reported that the exogenous infusion of
angiotensin II causes increased insulin sensitivity in
both nondiabetic and non–insulin-dependent diabetes
mellitus patients,34–37 possibly through an increase in
muscle blood flow, although angiotensin II itself ap-
ppears to have a direct biochemical effect on insulin
sensitivity through various intracellular pathways.36
The mean plasma ACE concentration in DD subjects is
approximately twice that in II subjects. This suggests
that DD subjects have more angiotensin II than ACE-II
subjects, but there is generally no difference in blood
pressure among the ACE genotypes. Thus, ACE levels
or activities, although having little effect on systemic
blood pressure, might affect the distribution of blood
flow within the microcirculation of skeletal muscle to
produce a net increase in the perfusion of insulin-
sensitive fibers. Angiotensin II-mediated blood flow in
skeletal muscle might be changed by the infusion of
angiotensin II,37 which is also regulated by the num-
ber of angiotensin II receptors and the distribution of
angiotensin type 1 and type 2 receptors. Therefore,
these basic data might also explain the divergent effect
of the I allele on insulin resistance in different study
populations.

ACE inhibitors have been shown to improve insulin
sensitivity,12–14 and this effect may be influenced by
bradykinin. The vasodilatory properties of bradykinin
operate through prostacyclins and nitric oxide, and
are not potentiated by angiotensin II receptor (ATR)
antagonism. However, a recent study showed that
ATR antagonists also improved insulin sensitivity.38,39
Therefore, further careful studies are needed to clarify
this point.

Patients with the DD genotype had higher concen-
trations of serum triglyceride levels than II patients in
our study (Table 2). Nagi et al32 reported that plasma
ACE levels were weakly but significantly associated
with plasma triglyceride levels, without being associ-
ated with the ACE genotype, in Pima Indians. Assum-
ing that ACE-DD subjects have higher plasma ACE
levels than those with the II genotype, our observa-
tions partly agree with their findings, but higher tri-
glyceride levels in DD were not supported by other
studies.30,31

We previously reported that the plasma fibrinogen
level was an independent indicator of the severity of
coronary atherosclerosis by a multivariate analysis.40
Patients with the II genotype had higher concentra-
tions of plasma fibrinogen than ID patients in this
study (Table 2). To date, insulin resistance or the pre-
cursors of insulin have been shown to directly stimu-
late plasminogen activator inhibitor type 1 synthe-

sis41,42; however, no significant relationship between
the ACE I/D genotype and parameters of fibrinolysis
or coagulation has been reported.6,31,43

In conclusion, the D allele was not associated with
insulin resistance; thus, angina pectoris patients with
the DD and ID genotype were associated with a sig-
nificantly lower risk of insulin resistance than those
with the II genotype.
REFERENCES


