The D Allele of the Angiotensin-Converting Enzyme Insertion/Deletion (I/D) Polymorphism is a Risk Factor for Type 2 Diabetes in a Population-Based Japanese Sample

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Abstract. The association of the ACE gene I/D polymorphism with type 2 diabetes (DM) was examined in a population-based Japanese sample. A total of 902 individuals (490 females and 412 males, age 58.8 ± 12.2 yr) from a cohort population ($n = 3,706$) of the Funagata diabetes study were divided into three groups according to genotype: D/D ($n = 104$), I/D ($n = 436$) and I/I ($n = 362$). Chi-square test and ANOVA were used for association studies and to assess the differences in the traits' values, respectively. More individuals with the genotypes D/D and I/D were diabetic (8.7% and 4.1%, respectively) than those with the genotype I/I (2.8%, $p = 0.008$ and $p = 0.032$, respectively). The genotype D/D was a risk factor for DM (relative risk (RR) 3.13, 95% CI 1.31–7.51), and also for DM and IGT (RR 1.78, 95% CI 1.14–2.76). Multiple logistic regression analysis also showed that the genotypes with the D allele were risk factors for DM and IGT even when adjusting for age, sex, hypertension and serum total cholesterol levels (odds ratio 1.49, 95% CI 1.01–2.21). The D allele of the ACE gene I/D polymorphism is a risk factor for DM.

Key words: ACE, I/D polymorphism, Diabetes, Population-based study, Relative risk

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THE angiotensin-converting enzyme (ACE) plays an important role in the renin-angiotensin system, by generating vasoconstrictor angiotensin II and degrading vasodilator kinins [1]. Changes of serum ACE levels thus seem influence the renin-angiotensin system, which consists of endocrine (or circulating) and local systems. A commonly occurring 287-bp insertion (I)/deletion (D) polymorphism in intron 16 of the ACE gene accounts for a large portion of the variance in serum ACE levels [2]. Subjects with the genotype D/D displayed the highest mean levels of serum ACE, those with the genotype I/I displayed the lowest, and those with the genotype I/D displayed intermediate levels.

Therefore, this ACE gene polymorphism has been expected to be associated with various diseases related to the renin-angiotensin system. Indeed, the D allele of the polymorphism has been reported to be associated with diseases that influenced by the endocrine system, such as diabetic nephropathy [3–9], coronary artery disease [8, 10–12], arterial wall thickness [13], arterial stiffness [14] and hypertension [11, 15].

In addition to the endocrine system, local systems exist in many tissues including skeletal muscle [16] and adipose tissue [17]. The local skeletal-muscle renin-angiotensin systems may modify the use of substrate through a kallikrein-kinin system, where low ACE (namely, kininase II) activity leads to an increased glucose uptake during exercise [18]. Furthermore, low ACE activity (ACE-inhibition) has been reported to increase skeletal-muscle glucose uptake, insulin sensitivity, glycogen storage, glucose transporter GLUT-4 synthase activity and hexokinase activ-

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ity [19]. The local adipose renin-angiotensin systems may alter substrate mobilization from fat stores. Low ACE activity has been reported to increase insulin-stimulated hexone transport in adipocytes [20] and insulin suppression of non-esterified fatty-acid flux [21]. Taken together, these findings seem to indicate that low ACE activity is advantageous for glucose metabolism. Inversely, high ACE activity, namely the ACE genotype D/D, seems to be disadvantageous for glucose metabolism or to be a risk factor for impaired glucose metabolism or DM. Therefore, we examined the association of the ACE gene I/D polymorphism with DM in a large population-based Japanese sample.

Materials and Methods

Subjects

The Funagata diabetes study is a population-based study to clarify the risk factors, related conditions, and consequences for DM from an epidemiological point of view [22]. In Funagata, an agricultural area located about 400 km north of Tokyo, the population aged over 35 years was 4,183 in 1995. Individuals ($n = 377$) with cerebrovascular diseases or other disabilities who were unable to attend the study were excluded. One hundred residents who had been identified by public health nurses and through contacts with outpatient clinics as having medication for diabetes were also excluded. Therefore, the number of residents registered for the study was 3,706. From 1995 to 1997, 2,013 residents attended the study. Among them, 902 were enrolled for a genetic analysis for the ACE I/D polymorphism; the participation rate for this genetic analysis was 44.8%. This study was approved by the Ethical Committee of the Yamagata University School of Medicine. Written informed consent to participate in this study was obtained from the subjects involved. The mean age ($\pm$ SD) and the sex ratio (female/male) of the study group were 58.8 ± 12.2 and 490/412, respectively. Glucose tolerance was diagnosed according to the 1985 WHO criteria [23]. The numbers of individuals with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and DM were 750, 115 and 37, respectively.

Clinical traits examined

Along with the genetic analysis, the following traits were analyzed: height, body weight, 75-g oral glucose tolerance test (OGTT), HbA1c, waist circumference, hip circumference, waist-to-hip ratio, body mass index (BMI), percent body fat, systolic blood pressure, diastolic blood pressure, total serum cholesterol, serum triglyceride, and serum HDL cholesterol. Percent body fat was assessed based on the principles of bioelectrical impedance [24]. Hypertension was diagnosed if the subjects had a systolic blood pressure of ≥ 160 mmHg or a diastolic blood pressure of ≥ 95 mmHg or was undergoing medical treatment for hypertension.

Genetic analysis

Genomic DNA was isolated from peripheral blood leukocytes by proteinase K and the phenol/chloroform extraction procedure. The genotyping for the ACE I/D polymorphism was performed by polymerase chain reaction-restriction fragment length polymorphism analysis as previously described [25]. The study population was divided into three groups according to genotype: D/D ($n = 104$), I/D ($n = 436$) and I/I ($n = 362$). Mean age ($\pm$ SD) and sex ratio (female/male) of the groups (D/D, I/D and I/I) were 58.3 ± 12.1 and 59/45, 58.8 ± 12.4 and 233/203, and 57.4 ± 11.9 and 198/164, respectively. No statistical differences in age or sex ratio were observed among the groups.

Statistic analysis

Chi-square tests were performed for the association studies. Data are given as the means $\pm$ SD. The statistical significances of the differences of the trait values between two groups (D/D vs. I/I or I/D vs. I/I) were assessed by analysis of variance (ANOVA). Scheffe's F test was used for *post hoc* analysis. Scheffe's F test generates confidence intervals (CIs) that are quite wide, and thus has less statistical power to detect differences than do the other *post hoc* tests. Therefore, the Scheffe's F test is considered to be suitable to make elaborate comparisons, or to calculate contrast. Multiple logistic regression analysis was used to determine the independent association of the genotypes with the D allele (namely, genotypes D/D and I/D), age, sex, hypertension and serum total cholesterol

levels with DM or DM and IGT. For this analysis, the number of DM patients did not seem to be large enough. Therefore, DM and IGT were treated together for the analysis. A value of $p < 0.05$ was accepted as statistically significant.

Results

The distribution of the genotypes defined by the ACE I/D polymorphism was examined. The frequencies of the D and I alleles were 35.7% and 64.3%, respectively, and the frequencies of the genotypes D/D, I/D and I/I were 11.5%, 48.3% and 40.1%, respectively. The genotypes were found to be in Hardy-Weinberg equilibrium. Allelic and genotype frequencies were in concordance with other observations in Japan (3, 4, 9, 10); therefore, our study population seemed to be genetically similar to others reported in Japan.

The associations of the genotype with DM is shown in Table 1. More individuals with the genotypes D/D (8.7%, $p = 0.008$) and I/D (4.1%, $p = 0.032$) were diabetic than those with the genotype I/I (2.8%). There were no differences between the genotypes D/D and I/D in terms of the frequencies of diabetic individuals ($p = 0.235$). The genotypes D/D and ID were associated with DM. Relative risk (RR) of the genotypes for DM, or DM and IGT can be estimated here, since this is a population-based study, or cross-sectional study. The RRs of the genotypes with the D allele (namely, the genotypes D/D and D/I), D/D and D/I for DM were 1.81 (95% CI 0.89–3.69), 3.13 (95% CI 1.31–7.51) and 1.49 (95% CI 0.70–3.20), respectively. However, multiple logistic regression analysis to reveal the independent association of the genotypes with the D allele, BMI, sex, hypertension and serum total cholesterol

levels with DM did not show a significant association of the genotypes (odds ratio (OR): 1.75 (95%CI: 0.820–3.723), $p = 0.148$; R^2 of the test, 0.096). We speculated that the number of DM patients in this study was not large enough to provide a statistical power that was strong enough to detect the association. Therefore, we treated DM and IGT together to enlarge the case number and calculated the RRs of the genotypes for DM and IGT. We believe the genetic background of IGT to be similar to that of DM, although the extent of metabolic abnormalities is different between DM and IGT. The RRs of the genotypes with the D allele, D/D and D/I for DM and IGT were 1.50 (95% CI 1.09–2.06), 1.78 (95% CI 1.14–2.76) and 1.43 (95% CI 1.03–1.99), respectively. Furthermore, multiple logistic regression analysis to reveal the independent association with DM and IGT also showed the significant associations of the genotypes with the D allele, age (1 year), BMI (1 kg/m²) and serum total cholesterol (10 mg/dl) (R^2 of the test, 0.115) (Table 2). No significant association was found for sex or hypertension (Table 2). These results indicate that the D allele of the polymorphism is an independent risk factor for DM or for DM and IGT.

As described in material and methods, the traits related to diabetes, obesity, hypertension and dyslipidemia were measured. Individuals with the genotype D/D had higher 2-hr plasma glucose levels (122.0 ± 58.7 vs. 106.8 ± 34.4 , $p = 0.005$) than those with the genotype I/I (Table 3). The two groups showed no significant differences in the other traits examined (Table 3). No trait was different between the individuals with the genotypes I/D and I/I, although the 2-hr plasma glucose levels tended to be higher in the individuals with the genotype I/D than in those with the genotype I/I (113.6 ± 42.5 vs. 106.8 ± 34.4 , $p = 0.073$). Namely, the traits related to obesity, insu-

Table 1. Frequency of DM and the ACE gene I/D polymorphism

	Genotypes		
	D/D*	I/D*	I/I
DM	9 (8.7)	18 (4.1)	10 (2.8)
IGT	15 (14.4)	63 (14.4)	37 (10.2)
NGT	80 (76.9)	355 (81.4)	315 (87.0)

Data are N(%). * indicates statistical significance

Frequency of DM in the genotype D/D and I/D were significantly different from that in the genotype I/I ($p = 0.008$ and 0.032 , respectively)

Table 2. Risk factors for DM and IGT when adjusting for clinical traits by multiple logistic regression analysis

Variables	odds ratio	95% CI	p
ACE I/D polymorphism (D allele)	1.49	1.01–2.21	0.044
Age (1 year)	1.07	1.05–1.09	<0.001
Sex (Female)	0.73	0.50–1.06	0.099
BMI (1 kg/m ²)	1.11	1.04–1.18	0.001
Hypertension	1.37	0.92–2.04	0.123
Serum total cholesterol (10 mg/dl)	1.07	1.01–1.13	0.023

CI indicates confidence interval.

Table 3. Effect of ACE gene I/D polymorphism

Parameter	Genotypes				
	D/D		I/D		I/I
	Value	P	Value	P	Value
Gender (W/M)	59/45		233/203		198/164
Age (yr)	58.3 ± 12.1	0.797	58.8 ± 12.4	0.312	57.4 ± 11.9
Height (cm)	155.8 ± 8.9	0.997	155.6 ± 8.7	0.912	155.8 ± 9.1
Body weight (kg)	58.5 ± 10.1	0.615	57.1 ± 9.7	0.847	57.5 ± 10.2
Fasting plasma glucose (mg/dl)	95.9 ± 14.2	0.236	95.4 ± 12.3	0.107	93.6 ± 11.5
2 hr plasma glucose (mg/dl)	122.0 ± 58.7	0.005*	113.6 ± 42.5	0.073	106.8 ± 34.4
Fasting serum insulin (mU/ml)	4.87 ± 3.26	0.792	4.91 ± 4.03	0.482	4.61 ± 2.88
HbA1c (%)	5.51 ± 0.62	0.161	5.45 ± 0.54	0.359	5.40 ± 0.44
Waist circumference (cm)	79.6 ± 9.02	0.575	78.6 ± 8.85	0.997	78.6 ± 8.91
Hip circumference (cm)	92.6 ± 5.96	0.843	91.8 ± 5.71	0.671	92.2 ± 5.91
Waist to hip ratio	0.860 ± 0.074	0.594	0.855 ± 0.071	0.838	0.852 ± 0.070
Body mass index (kg/m ²)	24.05 ± 3.03	0.394	23.52 ± 3.09	0.967	23.58 ± 3.11
Percent body fat (%)	27.07 ± 6.44	0.399	25.79 ± 6.83	0.862	26.05 ± 6.73
Systolic blood pressure (mmHg)	128.8 ± 16.2	0.151	126.4 ± 18.0	0.575	125.1 ± 16.8
Diastolic blood pressure (mmHg)	74.9 ± 9.20	0.962	74.7 ± 9.85	0.990	74.6 ± 10.0
Total cholesterol (mg/dl)	210.8 ± 34.9	0.826	205.3 ± 35.7	0.465	208.4 ± 34.8
Triglyceride (mg/dl)	137.7 ± 177.2	0.192	111.5 ± 80.3	0.765	116.9 ± 97.6
HDL cholesterol (mg/dl)	57.6 ± 14.1	0.732	57.3 ± 14.2	0.280	58.9 ± 15.1
HOMA-IR	1.16 ± 0.81	0.742	1.19 ± 1.06	0.287	1.08 ± 0.74

Values are mean ± SD. P values compared subjects carrying D/D or I/D genotype with subjects carrying I/I genotype.

lin resistance, hypertension or dyslipidemia were not different among the groups.

Discussion

The genotypes D/D and I/D were significantly associated with DM. The associations of these genotypes with DM complications or DM-related conditions have been reported in many case-control studies [3–10]. However, an association of the genotype with DM *per se* has not been clearly shown so far. In the present study, we determined the association of the genotype with DM. The relatively large number of our subjects (902 individuals) and the fact that our subjects were from a community (namely, no stratification was expected in the study population) was expected to increase the power to reveal the association. Furthermore, multiple logistic regression analysis revealed that the genotypes with the D allele were risk factors for DM and IGT independently from age, sex, BMI and other clinical traits. The genotype D/D seemed to be a stronger risk factor than the genotype I/D (RR: 1.78 vs 1.43). These findings indicated that the D

allele of the polymorphism was a risk factor for DM, and that the D allele contributed to the pathogenesis of DM in a dosage dependent manner.

We conjectured that the increased ACE activity in the individuals with the D allele may lead to impaired glucose metabolism or insulin resistance and eventually to DM. However, we could not find any evidence to support this idea in this study. The traits related to insulin resistance such as fasting serum insulin levels and HOMA-IR, were not significantly different among the individuals with the genotypes D/D, I/D, and I/I. Thus, at this point the mechanisms involved in the association of the D allele with the increased prevalence of DM are not clear. The increased ACE activity in the local renin-angiotensin system might be more responsible for the association than that in the systemic renin-angiotensin system. Thus, the traits related to the systemic renin-angiotensin system, or the serum levels of ACE, might not be clearly different among the study groups. It seems also possible that the traits used here such as fasting serum insulin levels and HOMA-IR were not appropriate to distinguish the differences in insulin resistance among the groups, since Japanese seem to more prone to become incompetent

at secreting insulin to overcome existing insulin resistance. The number of the individuals with the genotype D/D (11.5%) might not be large enough to reveal the differences in the traits as significant. In addition, it should be noted that the ACE I/D polymorphism may not be the causative variation but rather merely the genetic marker that is in linkage disequilibrium with other causative variations. Indeed, Rieder *et al.* found 78 variations of the gene, 17 of which were in absolute linkage disequilibrium with the I/D polymorphism reported here [26]. Therefore, some of these 17 variations may be causative. If so, an association study with such gene variations may reveal the association of the gene with DM to be even more significant.

The association of the D allele with the increased prevalence of DM was found in a dosage dependent manner in this study. This result seemed to be in good agreement with the findings of the association of the administration of an ACE inhibitor (namely, the decrease of ACE activity) with lower rates of new diagnosis (or the incidence) of DM in high-risk individuals [27, 28]. The D allele, which is responsible for increased ACE activity, seemed to be responsible for the increase prevalence of DM.

In conclusion, the D allele of the ACE gene I/D polymorphism was shown to be associated with DM in a population-based Japanese sample.

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