Brief Genetics Report

Insertion/Deletion Polymorphism of the ACE Gene Is Associated With Type 2 Diabetes

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In an attempt to examine the role of an ACE gene insertion/deletion (I/D) polymorphism in type 2 diabetes, we conducted a case-control association study among 132 couple-pairs from northern China. The genotype frequencies for II, ID, and DD were 39.8, 39.8, and 20.3%, respectively, in the case group and 44.8, 44.8, and 10.4% in the control group. The DD frequency was significantly higher in the case group than in the control group ($\chi^2_1 = 4.77$, $P = 0.03$), suggesting that the DD genotype is associated with an increased susceptibility to type 2 diabetes in our study population. *Diabetes* 51: 1986–1988, 2002

Type 2 diabetes is a complex disorder accounting for ~90–95% of all diabetes syndromes. Despite numerous reports suggesting a substantial genetic contribution to the susceptibility of type 2 diabetes, no major susceptibility genes have been identified so far (1, 2). ACE, a key enzyme in the renin-agiotensin system, catalyzes the conversion of angiotensin I to angiotensin II in liver and inactivates bradykinin in many tissues. ACE insertion/deletion (I/D) polymorphism, characterized by the presence (insertion) or absence (deletion) of a 287-bp *Alu*I-repeat sequence inside intron 16, has been suggested to be associated with coronary heart disease and nephropathy in type 2 diabetic patients (3–9). Association studies of ACE I/D polymorphism and type 2 diabetes in various populations have yielded conflicting results (10–13). Here, we report a case-control association study of ACE I/D polymorphism and type 2 diabetes in a Chinese population.

We tested the relationship between ACE I/D polymorphism and type 2 diabetes in 132 Chinese spousal case-control pairs, each pair consisting of a type 2 diabetes proband and his/her nondiabetic spouse. Every proband met the World Health Organization criteria for type 2 diabetes and had at least one first-degree family relative (parent or sibling) with type 2 diabetes. Each nondiabetic spouse control had to be free from any family history of type 2 diabetes and had to have normal glucose tolerance during a 75-g oral glucose tolerance test (OGTT). The phenotypic characteristics of the diabetic and spousal control subjects are summarized in Table 1. Age and sex were well matched between the case and the control groups, whereas the average BMI, waist-to-hip ratio, blood pressure, and serum concentrations of insulin, C-peptide, total cholesterol, and triglycerides were significantly higher in the case subjects. The genotype frequencies of ACE gene I/D polymorphism were shown in Table 2.

The D allele frequency was 40.2 and 32.8% in the case and the control groups, respectively, which is in line with previous reports of 29.3–41.6% frequencies in other Asian populations (5,10,11,13) and is much lower than the 52–57% frequencies reported in Caucasian populations (12,14,15). The observed genotype distribution was in Hardy-Weinberg equilibrium in control subjects ($\chi^2_1 = 0.03$, $P = 0.96$) and was marginally deviated from the equilibrium in case subjects ($\chi^2_1 = 3.77$, $P = 0.05$). A significantly higher count of DD genotype was observed in case than in control subjects ($\chi^2_1 = 4.77$, $P = 0.03$), suggesting an increased risk of type 2 diabetes for DD genotype in the study population. Among these 264 subjects collected, 250 (94.7%) were Northern Han Chinese, 6 (2.3%) were Hui Chinese, 5 (1.9%) were Man Chinese, and 3 (1.1%) were from other minority nationalities. Because population admixture can lead to spurious associations (16), we restricted our analysis to only the 250 Northern Han subjects to avoid the confounding effects due to genetic heterogeneity. Genotype data were available for 239 subjects, and the DD genotype was found to be significantly higher among case (20%) than among control (11%) subjects ($\chi^2_1 = 3.98$, $P = 0.046$). We also limited our analysis to the 120 Han-Han case-spouse pairs only. Genotype data were available for 230 subjects, and the results remained essentially the same (DD vs. II/ID, $\chi^2_1 = 3.67$, $P = 0.055$). Because complete genotype and phenotype data were available for a total of 110 Han-Han case-spouse pairs, we used the logistic regression model, adjusting for the confounding effects of age, sex, BMI, and cigarette smoking, and we observed that the DD genotype was significantly associated with an increased risk for type 2

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OGTT, oral glucose tolerance test.

TABLE 1
Characteristics of type 2 diabetic case and control subjects

Variables	Control subjects	Case subjects
n	132	132
Age (years)	53.7 ± 11.2	53.2 ± 10.0
Sex (M/F)	67/65	65/67
BMI (kg/m^2)	24.8 ± 2.8	27.2 ± 4.4*
Waist-to-hip ratio	0.85 ± 0.09	0.88 ± 0.06*
Fasting blood glucose (mmol/l)	4.6 ± 0.6	12.4 ± 4.4*
2-h blood glucose (mmol/l)	5.8 ± 1.2	15.8 ± 4.8*
Fasting insulin (μU/ml)	14.3 ± 6.9	18.4 ± 13.2*
2-h insulin (μU/ml)	57.5 ± 39.4	58.2 ± 50.0
Fasting serum C-peptide (pmol/ml)	0.71 ± 0.26	0.85 ± 0.40*
2-h serum C-peptide (pmol/ml)	2.35 ± 0.81	2.08 ± 1.06
LDL cholesterol (mmol/l)	3.00 ± 0.87	3.15 ± 0.93
HDL cholesterol (mmol/l)	1.28 ± 0.28	1.23 ± 0.39
Total cholesterol (mmol/l)	5.02 ± 0.91	5.33 ± 1.15†
Triglycerides (mmol/l)	1.42 ± 0.88	1.94 ± 1.57*
Systolic blood pressure (mmHg)	126.4 ± 18.6	133 ± 19.8*
Diastolic blood pressure (mmHg)	81.3 ± 10.6	83.4 ± 9.1*

Data are means ± SD. *$P < 0.01$; †$P < 0.05$. Comparisons were made using Student's *t* test (for continuous variables).

diabetes (adjusted odds ratio 3.08, 95% CI 1.22–7.75, $P = 0.017$).

Several previous studies reported that individuals with the ACE gene DD genotype were more insulin sensitive and were more likely to have lower insulin response to oral glucose loading than those with the ID/II genotype in both type 2 diabetic patients and nondiabetic populations (14,17–19). We found similar trends in the control subjects: individuals with the ACE gene DD genotype had lower insulin levels (fasting insulin 10.9 ± 2.9 μU/ml, OGTT 2-h insulin 43.1 ± 16.3 μU/ml) than individuals with the II/ID genotype (fasting insulin 14.6 ± 7.1 μU/ml, OGTT 2-h insulin 58.9 ± 40.8 μU/ml), although the difference was not statistically significant.

Although the I/D polymorphism is in the intronic region of the ACE gene, many studies showed that the DD genotype is strongly associated with increased plasma or serum ACE levels (3,20,21). The relation of this polymorphism with type 2 diabetes has been explored in several previous studies, but their findings could not be reconciled (10–13). In this report, we observed a significant association between the DD genotypes and type 2 diabetes, which were essentially unchanged after the exclusion of minority subjects. In light of the robustness of our results and the merit of the case-spouse design, which may have greatly lessened potential confounding effects due to different exposure levels to environmental or dietary factors, the current investigation could provide new evidence regarding the role of the ACE gene in the pathogenesis of type 2 diabetes, which may have significant clinical implications.

TABLE 2
The genotype frequencies of ACE gene I/D polymorphism*

	N	II	ID	DD
Case subjects	128	51 (0.398)	51 (0.398)	26 (0.203)
Control subjects	125	56 (0.448)	56 (0.448)	13 (0.104)

Data are *n* (frequency). *DD vs. II/ID: $\chi^2_1 = 4.77$, $P = 0.03$.

RESEARCH DESIGN AND METHODS

Study population and DNA sample preparation. A total of 132 spousal case-control pairs were ascertained from local hospitals in Beijing and Shenyang, two metropolitan cities in northern China. Each spouse pair consisted of *1*) a type 2 diabetic patient meeting the World Health Organization criteria and having at least one diabetic first-degree relative and *2*) the proband's spouse, who had normal glucose tolerance during a 75-g OGTT. A volume of 10 ml of venous blood was collected from each subject after an overnight fast for DNA preparation and for measurements of serum insulin, C-peptide, glucose, total cholesterol, triglycerides, HDL, and LDL. After the fasting blood samples were drawn, all subjects except the patients whose fasting blood glucose were higher than 11.1 mmol/l were given a 75-g oral glucose challenge. Blood was collected 2 h later for determination of blood glucose, insulin, and C-peptide. DNA was extracted from leukocytes using Puregene DNA isolation kits (Gentra Systems, Minneapolis, MN) as previously described (22). This study has been approved by the institutional review boards of the Beijing Medical University and the Harvard School of Public Health, and all study subjects gave informed consent. All procedures were in accordance with institutional guidelines.

Genotyping method of the ACE I/D polymorphism. Sequences flanking the ACE I/D polymorphism were PCR-amplified from genomic DNA using a pair of oligonucleotide primers: 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and 5′-GATGTGGCCATCACATTCGTCAGAT-3 (BioBasic, Markham, Canada). The PCR was carried out in 10 μl of 10 mmol/l Tris-HCl, 50 mmol/l KCl, 2.0 mmol/l $MgCl_2$, 200 μmol/l of each of the four deoxynucleotides, 1 μmol/l each of the primers, and 0.4 units *Taq* polymerase (Qiagen, Valencia, CA) on a PTC-225 thermal cycler (MJ Research, Waltham, MA). After an initial denaturation at 94°C for 3 min, the DNA was amplified by 30 PCR cycles of denaturation at 94°C for 30 s, annealing at 58°C for 45 s, and extension at 68°C for 45 s, followed by a final extension at 68°C for 7 min. PCR products were separated and sized by electrophoresis on a 2% agarose gel. The insertion allele (I) was detected as a 490-bp band, and the deletion allele (D) was visualized as a 190-bp band. All of the samples were genotyped twice independently in the molecular genetic laboratory of the Center for Ecogenetics and Reproductive Health of Beijing University. The genotype results were scored by two independent researchers without knowledge of the case/control status of each study individual. Overall, PCR failed in seven control and four case subjects. Genotyping was conducted twice, and the concordance rate of the two independent genotyping assays was 99%.

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