Interactions Among Related Genes of Renin-Angiotensin System Associated With Type 2 Diabetes

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OBJECTIVE — To explore the association between epistasis among related genes of the renin-angiotensin system (RAS) and type 2 diabetes.

RESEARCH DESIGN AND METHODS — Gene polymorphisms were genotyped in 394 type 2 diabetic patients and 418 healthy control subjects in this case-control study. We used the multifactor dimensionality reduction method to identify gene-gene interactions.

RESULTS — No single locus was associated with type 2 diabetes, except for the insert/deletion (I/D) polymorphism of the *ACE* gene in female subjects. In multi-locus analyses, in male subjects the model of rs2106809 (*ACE2*), rs220721 (*Mas*), rs699 (*AGT*), and I/D (*ACE*) was significant ($P = 0.043$). This combination was associated with a 4.00 times (95% CI 2.51–6.38; $P < 0.0001$) greater prevalence of type 2 diabetes. In female subjects, the model of rs2106809 (*ACE2*), I/D (*ACE*), and rs1403543 (*AGTR2*) was significant ($P = 0.012$). This three-locus combination was associated with a 2.76 times (1.91–3.97; $P < 0.0001$) greater prevalence of type 2 diabetes.

CONCLUSIONS — Interactions among RAS-related genes were associated with type 2 diabetes in a Chinese population.

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Over the past few years, a number of new genetic loci associated with type 2 diabetes have been uncovered based on genome-wide association scans. Investigations into gene-gene interactions, however, are uncommon because the method is computationally challenging (1). In type 2 diabetes, attempts to elucidate possible epistasis have provided only a few examples (2–4).

Recently, studies have supported the idea that using our understanding of biology, including that of cytokine networks and hormone systems, may help guide analysis of epistasis (5,6). Clinical evidence suggests that the renin-angiotensin system (RAS) is associated with the etiology of type 2 diabetes (7–9). However, the influence of genetic interaction within the RAS on type 2 diabetes susceptibility is still unknown. The aim of our study was to explore the contribution of epistasis among RAS-related genes.

RESEARCH DESIGN AND METHODS

— The study subjects were selected from an ongoing large-scale population-based cohort (10). Participants without previously known diabetes were selected from the 2,826 registered individuals. Written informed consent was obtained from each participant. Subjects' fasting plasma glucose (FPG) was obtained from our previous study, and the subjects with FPG >5.6 mmol/l performed a 75-g oral glucose tolerance test. Diabetes was diagnosed according to the 1999 World Health Organization criteria. All subjects in both groups were matched for blood pressure, serum creatinine, and age.

Eight single nucleotide polymorphisms (SNPs) from seven RAS-related genes were then assessed; these were as follows: rs699 (*AGT*), insert/deletion (I/D) polymorphism (*ACE*), rs2106809 and rs2074192 (*ACE2*), rs5186 (*AGTR1*), rs1403543 (*AGTR2*), rs220721 (*Mas*), and rs1799722 (*BDKRB2*). Detection was completed using a MassARRAY platform (Sequenom, San Diego, CA).

We used the multifactor dimensionality reduction (MDR) software 2.0-β (http://www.multifactordimensionalityreduction.org) to identify gene-gene interactions.

RESULTS

— A total of 394 unrelated type 2 diabetic patients and 418 healthy control subjects were enrolled in this case-control study. Demographic and clinical characteristics of the subjects are given in supplementary Table A1 in the online appendix available at http://care.diabetesjournals.org/cgi/content/full/dc10-0349/DC1. Age, blood pressure, and serum creatinine were comparable between the two groups. Because of the *ACE2* and *AGTR2* genetic presence in the X chromosome, the analysis was performed separately for male and female subjects.

Association analyses of male subjects

No single-locus analysis showed a significant association with type 2 diabetes (supplementary Table A2). According to the MDR analysis through five-locus comparisons, a significant interaction was observed for variant alleles in the following three loci: rs2106809 (*ACE2*), rs220721 (*Mas*), and I/D (*ACE*) (Table 1). This combination had the maximum cross-validation consistency of 10 that was significant at the 0.01 level of *P* value, as calculated using the sign test. The four-locus model of rs2106809 (*ACE2*), rs220721 (*Mas*), rs699 (*AGT*), and I/D (*ACE*) scored nine of cross-validation consistency that was significant at the 0.05 level of *P* value. The four-locus model was also significant in the 1,000 permutation test (Table 1). In the χ^2 test,

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Table 1—*MDR results of multi-locus interaction*

Best model	Testing balance accuracy	Cross-validation consistency	*P*	*P**
Male subjects				
Mas	0.4714	8	0.9893	0.804
Mas, BDKRB2	0.4759	5	0.8281	0.375
ACE2-A, ACE, Mas	0.6195	10	0.0107	0.172
ACE2-A, ACE, Mas, AGT	0.5624	9	0.0547	**0.043**
ACE2-A, ACE, Mas, AGT, BDKRB2	0.5399	6	0.1719	0.6010
Female subjects				
ACE2-B	0.5364	9	0.1719	0.516
ACE2-A, ACE	0.5567	9	0.1719	0.068
ACE2-A, ACE, AGTR2	0.5892	10	0.0010	**0.012**
ACE2-B, ACE, Mas, AGTR2	0.5319	6	0.1719	0.341
ACE2-A, ACE, Mas, AGT, AGTR2	0.5586	9	0.0547	0.148

**P* based on 1,000 permutations. Data in bold represent statistical significance. Genotypes: AGT (rs699) C/T; ACE (I/D); ACE2-A (rs2106809) G/A; ACE2-B (rs2074192) C/T; AGTR1 (rs5186) A/C; AGTR2 (rs1403543) A/G; Mas (rs220721) A/G; BDKRB2 (rs1799722) C/T.

the odds ratio (OR) of high-risk combination of four loci increased the risk of type 2 diabetes by 4.00 times (95% CI 2.51–6.38, $P < 0.0001$).

Association analyses of female subjects

The results of the single-locus analyses showed that only I/D from *ACE* was associated with type 2 diabetes ($P = 0.039$) (supplementary Table A2). According to the MDR analysis, the most significant combination was the three-locus model, rs2106809 (*ACE2*), I/D (*ACE*), rs1403543 (*AGT2R*), which had the maximum cross-validation consistency of 10 and was significant at the 0.012 level of *P* value, as calculated using permutation test. In the χ^2 test, the OR of high-risk combination of three loci increased the risk of type 2 diabetes by 2.76 times (95% CI 1.91–3.97, $P < 0.0001$).

The logistic regression model suggested a nonsignificant gene-gene interaction in a multiplicative manner in the male and female participants.

CONCLUSIONS — The results from our study evidenced that although main effects of the individual loci may not be observed, the interaction among RAS-related genes is directly correlated with the susceptibility of type 2 diabetes. It is thus possible that loci contribute to some complex diseases only by their interaction with other genes, although the main effects of the individual loci may be too small to be observed (11).

Identifying genes in multi-factorial diseases is difficult. There is no consensus as to the best strategy for detecting epistatic interactions in humans (12). In the present study, with MDR analysis we found interactions among RAS-related genes. These interactions make mechanistic sense because these genes are involved in the same biological pathways (13). However, the susceptibility interaction was not confirmed by the logistic regression analysis. A possible reason for these inconsistent results is that MDR did not detect the interaction defined by "deviation from the multiplicative" as in the logistic regression model. Only the significant results from MDR showed that the combination of different loci may increase or decrease the risk of disease (14). Based on the association OR of 1.3 (typical for type 2 diabetes) and allele frequency of 0.49, this study showed over 65% power to detect interactions of genes.

A limitation of our study is that by representing each gene locus with a single SNP, multiple association signals for a given gene might be missed (15). Furthermore, our findings need to be replicated in further studies with larger samples and different populations.

In conclusion, we were the first to show that the interactions among RAS-related genes are associated with type 2 diabetes.

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