Interactions among related genes of renin-angiotensin system associated with type 2 diabetes

Jin-Kui Yang, MD, PhD, Jian-Bo Zhou, MD, Zhong Xin, MD, Lei Zhao, MD, PhD, Mei Yu, BS, Jian-Ping Feng, RN, Hui Yang, MD, Ya-Hong Ma, MD

Department of Endocrinology, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China

Running title: Interactions among RAS genes and diabetes

Address correspondence and reprint requests to:
Professor Jin-Kui Yang,
E-mail: jinkui.yang@gmail.com

Additional information for this article can be found in an online appendix at http://care.diabetesjournals.org

Submitted 22 February 2010 and accepted 23 June 2010.

This is an uncopyedited electronic version of an article accepted for publication in Diabetes Care. The American Diabetes Association, publisher of Diabetes Care, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of Diabetes Care in print and online at http://care.diabetesjournals.org.
Objective— To explore the association between epistasis among related genes of the renin-angiotensin system (RAS) and type 2 diabetes (T2D).

Research design and methods— Gene polymorphisms were genotyped in 394 T2D patients and 418 healthy controls in this case-control study. We used the MDR method to identify gene-gene interactions.

Results— No single-locus was associated with T2D, except for the I/D polymorphism of ACE gene in female subjects. For 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 T2D. 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 T2D.

Conclusions— Interactions among RAS related genes were associated with T2D in a Chinese population.

Over the past few years, a number of new genetic loci associated with type 2 diabetes (T2D) have been uncovered based on genome-wide-association scan (GWAS). Investigations into gene-gene interactions, however, are uncommon as the method is computationally challenging (1). In T2D, 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, such as cytokine networks or 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 T2D (7-9). However the influence of genetic interaction within the RAS on T2D 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 blood pressure, serum creatinine and age matched.

Eight SNPs from seven RAS-related genes were then assessed, these were as follows: rs699 of the AGT, Insert/deletion polymorphism of the ACE, rs2106809 & rs2074192 of the ACE2, rs5186 of the AGTR1, rs1403543of the AGTR2, rs220721 of Mas and rs1799722 of BDKRB2. Detection was completed using a MassARRAY platform (Sequenom, San Diego, CA).

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

Results
A total of 394 unrelated T2D patients and 418
healthy controls 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. Age, blood pressure and serum creatinine between two groups were comparable. Because of the ACE2 and AGTR2 genetic presence in the X chromosome, the analysis was performed separately for male and female subjects.

**Male subjects association analyses.** No single locus analysis showed a significant association with T2D (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: ACE2 rs2106809, Mas rs220721 and ACE I/D (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 sign test. The four-locus model of rs2106809 (ACE2), rs220721 (Mas), rs699 (AGT) and I/D (ACE) scored 9 of cross validation consistency that was significant at the 0.05 level of P-value. The four-locus model was also significant in the 1000 permutation test (Table 1). In the \( \chi^2 \) test, the odd ratio (OR) of high-risk combination of four-locus increased the risk of the T2D by 4.00 times (95% CI 2.51 to 6.38, \( p<0.0001 \)).

**Female subjects association analyses.** The results of the single-locus analyses showed that only I/D from ACE was associated with T2D (\( p=0.039 \)) (supplementary Table A2). According to the MDR analysis, the most significant combination was the three-locus model, ACE2 rs2106809, ACE I/D, AGT2R rs1403543, 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 \( \chi^2 \) test, the OR of high-risk combination of three-locus increased the risk of the T2D by 2.76 times (95% CI 1.91 to 3.97, \( p<0.0001 \)).

The logistic regression model suggested a non-significant 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 T2D. It is thus possible that loci contribute to some complex diseases only by their interaction with other genes, whilst 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 T2D) 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 out (15). Furthermore, our findings need to be replicated in further studies with larger samples and different populations.

In conclusion, we first showed that the interactions among RAS related genes were
Interactions among RAS genes and diabetes

associated with T2D.

**Author Contributions**—J.K. Yang, researched data, contributed to discussion, reviewed/edited manuscript. J.B. Zhou, wrote manuscript. Z. Xin, researched data. L. Zhao, researched data. M. Yu, researched data. J.P. Feng, researched data. H. Yang, researched data. Y.H. Ma, researched data.

**ACKNOWLEDGEMENTS**

This work was supported by the National Natural Science Foundation of China (No.30671001 and No.30871887) and the National 863 Program of China (Grant 2006AA02A409) to Professor Jin-Kui Yang. The authors thank all the participants and staff in their study.

No potential conflicts of interest relevant to this article were reported.

**REFERENCES**


Table 1. MDR results of multi-locus interaction

<table>
<thead>
<tr>
<th>Best Model</th>
<th>Testing Balance</th>
<th>Cross-validation Consistency</th>
<th>P-value</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mas</td>
<td>0.4714</td>
<td>8</td>
<td>0.9893</td>
<td>0.804</td>
</tr>
<tr>
<td>Mas, BDKRB2</td>
<td>0.4759</td>
<td>5</td>
<td>0.8281</td>
<td>0.375</td>
</tr>
<tr>
<td>ACE2-A, ACE, Mas</td>
<td>0.6195</td>
<td>10</td>
<td>0.0107</td>
<td>0.172</td>
</tr>
<tr>
<td>ACE2-A, ACE, Mas, AGT</td>
<td>0.5624</td>
<td>9</td>
<td>0.0547</td>
<td>0.043</td>
</tr>
<tr>
<td>ACE2-A, ACE, Mas, AGT, BDKRB2</td>
<td>0.5399</td>
<td>6</td>
<td>0.1719</td>
<td>0.6010</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACE2-B</td>
<td>0.5364</td>
<td>9</td>
<td>0.1719</td>
<td>0.516</td>
</tr>
<tr>
<td>ACE2-A, ACE</td>
<td>0.5567</td>
<td>9</td>
<td>0.1719</td>
<td>0.068</td>
</tr>
<tr>
<td>ACE2-A, ACE, AGTR2</td>
<td>0.5892</td>
<td>10</td>
<td>0.0010</td>
<td>0.012</td>
</tr>
<tr>
<td>ACE2-B, ACE, Mas, AGTR2</td>
<td>0.5319</td>
<td>6</td>
<td>0.1719</td>
<td>0.341</td>
</tr>
<tr>
<td>ACE2-A, ACE, Mas, AGT, AGTR2</td>
<td>0.5586</td>
<td>9</td>
<td>0.0547</td>
<td>0.148</td>
</tr>
</tbody>
</table>

P-value* was based on 1000 permutations.
RAS related genes: AGT, angiotensinogen; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; AGTR1, angiotensin II type I receptor; AGTR2, angiotensin II type II receptor; BDKRB2, bradykinin receptor.
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/