Renin-Angiotensin System Gene Polymorphisms and Atrial Fibrillation

Chia-Ti Tsai, MD; Ling-Ping Lai, MD, PhD; Jiunn-Lee Lin, MD, PhD; Fu-Tien Chiang, MD, PhD; Juey-Jen Hwang, MD, PhD; Marylyn D. Ritchie, MS; Jason H. Moore, PhD; Kuan-Lih Hsu, MD, PhD; Chuen-Den Tseng, MD, PhD; Chiau-Suong Liau, MD; Yung-Zu Tseng, MD

Background—The activated local atrial renin-angiotensin system (RAS) has been reported to play an important role in the pathogenesis of atrial fibrillation (AF). We hypothesized that RAS genes might be among the susceptibility genes of nonfamilial structural AF and conducted a genetic case-control study to demonstrate this.

Methods and Results—A total of 250 patients with documented nonfamilial structural AF and 250 controls were selected. The controls were matched to cases on a 1-to-1 basis with regard to age, gender, presence of left ventricular dysfunction, and presence of significant valvular heart disease. The ACE gene insertion/deletion polymorphism, the T174M, M235T, G-6A, A-20C, G-152A, and G-217A polymorphisms of the angiotensinogen gene, and the A1166C polymorphism of the angiotensin II type I receptor gene were genotyped. In multilocus haplotype analysis, the angiotensinogen gene haplotype profile was significantly different between cases and controls ($\chi^2=62.5$, $P=0.0002$). In single-locus analysis, M235T, G-6A, and G-217A were significantly associated with AF. Frequencies of the M235, G-6, and G-217 alleles were significantly higher in cases than in controls ($P=0.000$, 0.005, and 0.002, respectively). The odds ratios for AF were 2.5 (95% CI 1.7 to 3.3) with M235/M235 plus M235/T235 genotype, 3.3 (95% CI 1.3 to 10.0) with G-6/G-6 genotype, and 2.0 (95% CI 1.3 to 2.5) with G-217/G-217 genotype. Furthermore, significant gene-gene interactions were detected by the multifactor-dimensionality reduction method and multilocus linkage disequilibrium tests.

Conclusions—This study demonstrates the association of RAS gene polymorphisms with nonfamilial structural AF and may provide the rationale for clinical trials to investigate the use of ACE inhibitor or angiotensin II antagonist in the treatment of structural AF. (Circulation. 2004;109:1640-1646.)

Key Words: arrhythmia • genetics • fibrillation • renin • angiotensin

The renin-angiotensin system (RAS) has been shown to be involved in many cardiovascular diseases, including myocardial fibrosis and hypertrophy in hypertensive heart disease,1 congestive heart failure,2 myocardial infarction,3 and cardiomyopathy.4 Recent reports suggest that atrial fibrillation (AF) is associated with activation of the RAS in the atria in humans5 and in a dog model of AF.6 Angiotensin II has been shown to trigger the mitogen-activated protein kinase pathway,5,6 which is responsible for the proliferation of fibroblasts and hypertrophy of cardiomyocytes.7 It has also been shown that inhibition of endogenous angiotensin II prevented atrial effective refractory period shortening during rapid atrial pacing in dogs.8 These results indicate that angiotensin II may be involved in the mechanism of atrial structural and electrical remodelings and that inhibition of the cardiac RAS by ACE inhibitors or angiotensin receptor antagonists may affect the pathophysiological substrate of AF and may offer a new therapeutic approach for AF.9–11

There have been several reports addressing the genetic control of familial lone AF.12–14 However, the genetic study of nonfamilial structural AF is scarce in the literature. On the basis of the aforementioned studies on RAS and AF, we hypothesized that RAS genes might be the susceptibility genes of nonfamilial structural AF and performed a genetic case-control study to demonstrate this. We completely genotyped 8 polymorphisms among the RAS genes, including the ACE gene insertion/deletion (I/D) polymorphism, the G-217A, G-152A, A-20C, G-6A, M235T, and T174M polymorphisms of the angiotensinogen (AGT) gene, and the A1166C polymorphism of the angiotensin II type I receptor gene (AT1R). The associations of these polymorphisms with cardiovascular studies have been reported15–20 except for the G-152A polymorphism. On the basis of our results from a transcriptional activity study of the AGT gene, we found that a more upstream promoter region, in addition to the core-promoter element 1 that contains the A-20C...
and G-6A polymorphisms, also plays a critical role in transcriptional control. Therefore, we also chose the G-152A and G-217A polymorphisms in this upstream region for the present study.

Because our genotype data were multilocus, the interactions between these loci or genes should be evaluated and addressed. Indeed, there is increasing evidence that epistasis or gene-gene interactions play an important role in determining an individual’s risk of complex-trait disease. However, the identification and characterization of gene-gene interactions have been limited by a lack of powerful statistical methods and a lack of large sample sizes. Several new strategies have been developed recently to solve these problems. Accordingly, we have adopted these methods in the present study to explore the multilocus effects of RAS genes on the development of nonfamilial structural AF.

**Methods**

**Study Population**

The study included 250 consecutive patients who were admitted to our adult cardiology ward with a history of AF. Patients with hyperthyroidism were excluded. Patients who were less than 65 years old and had no identifiable cause of AF (lone AF) were also excluded. None of the AF was familial. For every case patient, a matched control without a history of AF was selected from the same ward. Case and control patients were individually matched with regard to their gender, age (difference ≤5 years), presence of left ventricular dysfunction (ejection fraction <55%), and presence of significant valvular heart disease (at least moderate to severe). Hypertension and diabetes are known phenotypes associated with polymorphisms within RAS genes. Therefore, case and control patients were also matched as to these variables by frequency (group matching). After the cases and controls were matched, the clinical variables of the cases were comparable to those of the controls, except for mean left atrial size (Table 1). Cases had a significantly larger mean left atrium than controls (Table 1). Forty-five percent of the patients had significant valvular heart disease. More than 80% of the AF patients had persistent AF. The study protocols were reviewed and approved by the local institutional committee. All patients agreed to participate in the study, and verbal informed consent was obtained from each patient.

**Clinical Assessment**

The presence of AF was determined by taking the patient’s history, serial ECG, and/or ambulatory ECG monitoring. Patients with palpitations without ECG documentation were excluded from both patient and control groups. Transthoracic echocardiography was performed to measure left atrial and left ventricular dimensions and to detect significant valvular disease (defined as moderate to severe or severe valvular regurgitation or stenosis). Left ventricular mass was calculated with echocardiographic parameters and the Devereux formula.

**Identification of Diallelic Polymorphisms**

Genomic DNA was extracted by a nondenaturing method. DNA fragments were amplified by polymerase chain reaction (PCR). Genotyping of the ACE gene I/D polymorphism was performed as described previously. Genotyping of the AT1R gene A1166C polymorphism was performed with the PCR restriction fragment-length polymorphism method. For genotyping of AGT gene polymorphisms, we used mini-PCR direct sequencing as described previously.

**Statistical Methods**

For comparison of the baseline characteristics, between-group data were compared with the Student’s unpaired t test for continuous data and the χ² test for categorical data. With regard to the 6 polymorphisms within the AGT gene, because they were located on the same chromosome with short distances between each other, they probably did not segregate independently and had linkage disequilibrium (LD) between each other. Therefore, we first used haplotype analysis for these 6 polymorphisms to determine whether there were any specific haplotypes that were associated with AF. The methods of the expectation-maximization–based haplotype frequency estimation and permutation-based hypothesis-testing procedure were performed on the basis of the work of Fallin et al. After we obtained a significant haplotype profile analysis, we performed

<table>
<thead>
<tr>
<th>Table 1. Baseline Characteristics of the Patients</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Gender (male/female), n</td>
</tr>
<tr>
<td>Valvular heart disease</td>
</tr>
<tr>
<td>Body height, cm</td>
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<tr>
<td>Body weight, kg</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
</tr>
<tr>
<td>Left atrial dimension, mm</td>
</tr>
<tr>
<td>LVEDD, mm</td>
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<tr>
<td>LVESD, mm</td>
</tr>
<tr>
<td>LVEF, %</td>
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<tr>
<td>LV mass, g</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
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<tr>
<td>Smoking</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; LVEDD, left ventricular end diastolic dimension; LVESD, left ventricular end systolic dimension; LVEF, left ventricular ejection fraction; LV, left ventricular; SBP, systolic blood pressure; and DBP, diastolic blood pressure.
the individual haplotype analyses and single-locus analyses with multiple test correction for the probability value. For single-locus analyses, allele frequencies were calculated from the genotypes of the subjects. Differences in allele and genotype frequencies between cases and controls were compared with the χ² test or Fisher’s exact test. To avoid spurious associations, we used the permutation method to compare allele frequencies between cases and controls.27 The genotype-phenotype correlation was examined with additive, dominant, and recessive models with logistic regression. ORs and their 95% CIs were calculated. Because left atrial size was not balanced in cases and controls, we also performed the analyses with adjustment for left atrial size.

For evaluation of gene-gene interactions with a relatively small sample size but a large locus number, as in the present study, we used 2 methods. First, we used the multifactor-dimensionality reduction (MDR) method, developed by Ritchie et al.,23,29,30 to identify high-order gene-gene interactions. This method included a combined cross-validation/permutation-testing procedure that minimizes false-positive results by multiple examinations of the data. Cross-validation divides the data into a training set and a testing set. With 10-fold cross-validation, the data are divided into 10 equal parts, and the model is developed on 9/10 of the data (training set) and then tested on 1/10 of the remaining data (testing set). This is repeated for each possible 9/10 and 1/10 of the data, and the resulting 10 prediction errors are averaged. Second, we also used multilocus LD tests, proposed by Williams et al.,24,28 to evaluate gene-gene interactions.

A probability value ≤0.006 (0.05/64) was considered statistically significant after Bonferroni correction in the single-locus analyses. In the haplotype analysis and multilocus LD tests, the probability value was also corrected according to the number of tests performed.

We also performed subgroup analyses. All of the aforementioned statistical analyses were performed on patients with significant valvular heart disease (112 case-control pairs, \( n = 112 \times 2 = 224 \)) and nonvalvular heart disease (138 case-control pairs, \( n = 138 \times 2 = 276 \)), respectively.

**Results**

**Haplotypes of the AGT Gene and Their Association With AF**

Table 2 displays the results of 6-locus estimated haplotype frequency analyses for the AGT gene in cases and controls for the entire population. With 6 loci, there should be 2^6=64 haplotypes. However, because there were LDs in this small region of the AGT gene, some haplotypes did not exist or had very low frequencies, and we only listed haplotypes with frequency >0.01. The omnibus haplotype profile test was highly significant (\( \chi^2 = 62.5, P = 0.0002 \)), which indicated the overall haplotype frequency profile difference between cases and controls was significant, and thus there might be some disease-predisposing haplotypes in patients with AF.

Accordingly, in the individual haplotype analyses, we identified 3 haplotypes (AGAACC, AAAACC, and GGAACT) with significantly higher haplotype frequency in the cases than in the controls at the significance level \( P<0.05 \) by permutation tests (the results would not be significant if the stringent Bonferroni correction for probability value were used \( [P<0.05/64 \text{ for 64 individual haplotype analyses were performed}] \), except for the GGAACT haplotype \( [P=0.0002] \). All of these significant haplotypes had high haplotype frequencies (>0.01). The AAAACC and GGAACT haplotypes had ORs that indicated a large association effect (either >4 or <0.25; Table 2). The GGAACT haplotype had the lowest probability value (0.0002). When we analyzed the loci that composed these 3 haplotypes, we found that G-217A, G-152A, and M235T were the potential significant loci associated with AF.

In patients with valvular heart disease, the omnibus haplotype profile test was not significant (\( \chi^2 = 29.8, P = 0.098 \)), which could be the result of the decreased sample size after stratification. The GGAACT haplotype still had the lowest probability value (0.006) and had frequencies >0.01 and an OR <0.25 (0.2).

**Table 2. Haplotype Frequency Estimates of AGT Gene in Patients With AF and Controls and Significance Levels of Comparison From Permutation Tests**

<table>
<thead>
<tr>
<th>Haplotype*</th>
<th>Overall (n=500)</th>
<th>AF (n=250)</th>
<th>Controls (n=250)</th>
<th>OR</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>-217 -152 -20 -6 3889† 4072†</td>
<td>0.562 0.545 0.586 1.2 0.108</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G G A A C C</td>
<td>0.136 0.112 0.154 1.4 0.047</td>
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</tr>
<tr>
<td>G G A A C C</td>
<td>0.011 0.000 0.020 22.4 0.017</td>
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</tr>
<tr>
<td>G G C A C C</td>
<td>0.016 0.015 0.012 0.8 0.705</td>
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<td></td>
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</tr>
<tr>
<td>G A C A C C</td>
<td>0.014 0.008 0.020 2.5 0.171</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G G A G C C</td>
<td>0.022 0.029 0.013 0.4 0.080</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G G A A T C</td>
<td>0.043 0.041 0.047 1.1 0.744</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A G A A T C</td>
<td>0.011 0.005 0.016 3.3 0.231</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X G C A T C</td>
<td>0.046 0.058 0.034 0.6 0.083</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G A A C T</td>
<td>0.042 0.069 0.016 0.2 0.0002</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G G A G C T</td>
<td>0.060 0.069 0.053 0.8 0.328</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G G A G T T</td>
<td>0.011 0.015 0.004 0.3 0.107</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Log likelihoods: -1432.8 -737.1 -664.4 62.5§ 0.0002

*Haplotypes are not listed if all the estimated frequencies are 0.01 in patients with AF, controls, and overall population.
†C3889T dinucleotide polymorphism corresponds to T174M amino acid polymorphism, and T4072C corresponds to M235T.
‡P values based on 10 000 permutations.
§Likelihood ratio test statistic values for omnibus test.
In patients without valvular heart disease, the omnibus haplotype profile test was even more significant ($\chi^2=54.9, P=0.0001$). The GGAAC'T haplotype did not have the lowest probability value this time (0.005) but still had frequencies $>0.01$ and an OR $<0.25$ (0.2). The GGACGT haplotype had the lowest probability value (0.003) and also had frequencies $>0.01$ and an OR $<0.25$ (0.1). However, this haplotype did not have a significant probability value in patients with valvular heart disease or in the entire population.

**Single-Locus Analyses**

The results of the single-locus analyses for the entire population are shown in Table 3. The G-217A, G-6A, and M235T polymorphisms of the AGT gene were associated with AF with at least 1 significant probability value among the models of analysis. The M235T and G-217A polymorphisms were associated with AF in most models of the analyses, including the allele and genotype frequencies, additive model, and recessive (M235T) or dominant (G-217A) model analyses, whether corrected for left atrial size or not. The ORs were all $\leq 0.5$. These 2 loci were also found to be the candidate loci inferred from the haplotype analysis. Another candidate locus (G-152A) was not significant in this single-locus analysis.

In subgroup analyses for patients with valvular heart disease, the G-217A and M235T polymorphisms were associated with AF with at least 1 probability value $<0.05$ among the models, but no model was significant at $P\leq0.006$ (data not shown). The G-6A polymorphism was not associated with AF in this subgroup. In patients without valvular heart disease, the results were similar to those from the entire study population (data not shown).

**Gene-to-Gene Interaction**

**MDR Approach**

Table 4 summarizes the results obtained from MDR analysis for each number of loci evaluated for the entire population. One 3-locus model had a minimum prediction error of 37.26% and a maximum cross-validation consistency of 10.0 that was significant at the $<0.001$ level, as determined empirically by permutation testing. A 4-locus model also had a significant probability value (0.01). This 4-locus model consisted of the 3-locus model and an additional G-6A polymorphism that was also associated with AF in the single-locus analysis. However, this 4-locus model had a lower cross-validation consistency (8.4) and a higher prediction error (39.42%) than those in the 3-locus model. Therefore, we chose the 3-locus model as the best model.

In patients with valvular heart disease, MDR analysis revealed the same 4-locus model (T174M, M235T, G-6A, and I/D; $P=0.02$, prediction error 37.75%, cross-validation consistency 9.4). In patients without valvular heart disease, there were 3 models, all of which had probability values $<0.001$: the same 3-locus model and 4-locus model as in the entire population and a 5-locus model with an additional AT1 R polymorphism. The 3-locus model had the highest cross-validation consistency (9.5) and the lowest prediction error (34.79%). Therefore, we chose the 3-locus model as the best model.

**Multilocus LD Tests**

There were 247 possibilities of multilocus combination. The differences in LD pattern between cases and controls were evaluated. After multiple-test correction for probability values, there were 15 multilocus LDs that were found only in the cases and not in the controls, and 5 LDs that were found only in the controls and not in the cases (Table 5). These results indicated that there were multilocus and multigene interactions.

In the subgroup analyses, there were also LDs that were found exclusively in the cases or controls of both subgroups (data not shown). For patients with valvular heart disease, there were 12 multilocus LDs that were found exclusively in the cases and 9 LDs that were found exclusively in the controls. For patients without valvular heart disease, there were 4 multilocus LDs found exclusively in the cases and 6 LDs found exclusively in the controls. These results indicated that there were also multilocus and multigene interactions in both subgroups.

**Discussion**

Here we report for the first time associations between RAS gene polymorphisms and nonfamilial structural AF. Our findings provide the possibility that RAS genes are candidate genes not only for hypertension but also for nonfamilial structural AF.

**Possible Explanation of the Association**

We demonstrated that the M235 allele in exon 2 of the AGT gene, the G-6 and G-217 alleles in the promoter region of the AGT gene, and the corresponding haplotypes were associated with AF. A specific haplotype may be associated with higher AGT gene transcription activity. This higher transcription may cause a higher tissue angiotensin II concentration in the atrium under the stimulation of high atrial pressure, which subsequently activates the mitogen-activated protein kinase pathway and causes atrial fibrosis, conduction heterogeneity, and decreased atrial effective refractory period and provides the substrates for the development of AF. In our subgroup analyses, most of the results were similar in patients with valvular AF and with nonvalvular AF. This finding may imply that the final common pathway for the development of AF is the same, that is, stretching of the atria, regardless of the underlying causes of AF, such as mitral regurgitation or hypertension.

There is no functional significance of the M235T polymorphism. The association of the M235 allele with AF may be through its tight linkage with the G-6 allele. However, M235T was the most significant locus in the present study, even more significant than the G-6A locus. Therefore, we cannot rule out the possibility that M235T is linked to other loci with functional significance.

**Gene-to-Gene Interactions**

In the MDR analysis, we found an interaction between the T174M, M235T, and I/D polymorphisms. The T174M and I/D polymorphisms were not associated with AF in single-locus analyses. This is the evidence of epistasis: the effect of 1 gene may not be disclosed if the effect of another gene is not considered. This high-order gene-gene interaction could not be readily disclosed by conventional analytical methods. Furthermore, these 3 loci were located within 2 different
### TABLE 3. Distribution of Genotypes and Alleles in Patients With AF and Controls

<table>
<thead>
<tr>
<th>Locus</th>
<th>Genotypes and Alleles</th>
<th>AF (n=250)</th>
<th>Controls (n=250)</th>
<th>OR (95% CI), No Adjustment†</th>
<th>OR (95% CI), Adjustment for LA†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE gene</td>
<td>I/D</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>98/84</td>
<td>0.418</td>
<td></td>
<td>0.180</td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>98/105</td>
<td>0.9 (0.7–1.1)</td>
<td>0.7 (0.6–1.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DD</td>
<td>54/61</td>
<td>0.8 (0.5–1.1)</td>
<td>0.7 (0.4–1.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I:D</td>
<td>0.59:0.41</td>
<td>0.55:0.45</td>
<td>0.9 (0.6–1.3)</td>
<td>0.7 (0.4–1.1)</td>
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<tr>
<td>AT1R gene</td>
<td>A1166C</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>AA</td>
<td>228/223</td>
<td>0.692</td>
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</tr>
<tr>
<td></td>
<td>AC</td>
<td>21/25</td>
<td>0.8 (0.5–1.4)</td>
<td>0.9 (0.4–1.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>1/2</td>
<td>0.8 (0.4–1.4)</td>
<td>0.9 (0.5–2.0)</td>
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</tr>
<tr>
<td></td>
<td>A:C</td>
<td>0.95:0.05</td>
<td>0.94:0.06</td>
<td>0.5 (0.1–5.5)</td>
<td>0.6 (0.1–5.2)</td>
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<tr>
<td>AGT gene</td>
<td>G-217A</td>
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<td></td>
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<td></td>
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<td></td>
<td>GG</td>
<td>189/157</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>57/85</td>
<td>0.6 (0.4–0.8)‡</td>
<td>0.5 (0.3–0.8)‡</td>
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<tr>
<td></td>
<td>AA</td>
<td>4/8</td>
<td>0.5 (0.4–0.8)‡</td>
<td>0.5 (0.3–0.8)‡</td>
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<tr>
<td></td>
<td>G:A</td>
<td>0.87:0.13</td>
<td>0.80:0.20</td>
<td>0.5 (0.1–1.7)</td>
<td>0.5 (0.1–2.3)</td>
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<tr>
<td></td>
<td>G-152A</td>
<td></td>
<td></td>
<td>0.222</td>
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<tr>
<td></td>
<td>GG</td>
<td>234/226</td>
<td>0.115</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>16/22</td>
<td>0.6 (0.3–1.2)</td>
<td>0.7 (0.3–1.5)</td>
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</tr>
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<td>AA</td>
<td>0/2</td>
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<td>0.7 (0.3–1.6)</td>
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<td></td>
<td>G:A</td>
<td>0.97:0.03</td>
<td>0.95:0.05</td>
<td>§</td>
<td>§</td>
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<td>0.168</td>
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<td></td>
<td>AA</td>
<td>204/216</td>
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<td></td>
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<tr>
<td></td>
<td>AC</td>
<td>43/29</td>
<td>1.3 (0.8–1.9)</td>
<td>1.5 (0.9–2.5)</td>
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<tr>
<td></td>
<td>CC</td>
<td>3/5</td>
<td>1.4 (0.9–2.3)</td>
<td>1.9 (1.1–3.5)</td>
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</tr>
<tr>
<td></td>
<td>A:C</td>
<td>0.90:0.10</td>
<td>0.92:0.08</td>
<td>0.6 (0.1–2.5)</td>
<td>0.6 (0.1–3.1)</td>
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<tr>
<td></td>
<td>G-6A</td>
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<td></td>
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<tr>
<td></td>
<td>GG</td>
<td>14/4</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>GA</td>
<td>37/30</td>
<td>0.6 (0.4–0.9)</td>
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<td>AA</td>
<td>199/216</td>
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<td>0.3 (0.1–0.9)§</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G:A</td>
<td>0.13:0.87</td>
<td>0.08:0.92</td>
<td>0.6 (0.4–1.0)</td>
<td>0.8 (0.4–1.4)</td>
</tr>
<tr>
<td></td>
<td>T174M¶</td>
<td></td>
<td></td>
<td>0.237</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>193/202</td>
<td>0.143</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/M</td>
<td>47/44</td>
<td>1.3 (0.9–1.9)</td>
<td>1.1 (0.7–1.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M/M</td>
<td>10/4</td>
<td>1.2 (0.8–1.9)</td>
<td>1.1 (0.6–1.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T:M</td>
<td>0.87:0.13</td>
<td>0.90:0.10</td>
<td>2.5 (0.8–8.3)</td>
<td>1.4 (0.2–8.6)</td>
</tr>
<tr>
<td></td>
<td>M235¶</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M/M</td>
<td>9/4</td>
<td>0.05</td>
<td>0.3 (0.2–0.5)‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M/T</td>
<td>61/30</td>
<td>0.4 (0.1–1.4)‡</td>
<td>0.2 (0.1–1.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>180/216</td>
<td>0.4 (0.3–0.6)‡</td>
<td>0.3 (0.2–0.5)‡</td>
<td></td>
</tr>
</tbody>
</table>

LA indicates left atrial size.

*P values obtained based on \( \chi^2 \) test or Fisher’s exact test; the upper \( P \) value is for comparison of genotype frequencies, and the lower is for allele frequencies.

†ORs obtained by logistic regression; the top OR is for additive model, the middle for autosomal dominant model, and the bottom for autosomal recessive model.

‡ORs for recessive model could not be calculated because number of homozygotes is zero in patients with AF.

§P<0.006 (Bonferroni correction).

¶Diamino acid polymorphism.
chromosomes, which indicates that the interaction crossed chromosomal boundaries between the AGT and ACE genes. This interaction makes mechanistic sense, because these genes are involved in the same biological pathway.

Recently, Williams et al.\textsuperscript{24} used multilocus LD tests to evaluate gene-gene interactions for the genetic study of hypertension. In their report, many significant multilocus LDs, including many crossed chromosome loci, were found in hypertensive subjects, but none were found in healthy subjects. This finding indicates that epistasis among these loci is associated with hypertension. However, the data structure of the present study was very different from that of Williams et al.\textsuperscript{24} In their data, the cases consisted of patients with hypertension, and the controls were healthy individuals. However, control subjects in the present study did not consist of healthy individuals but rather of patients with diseases, such as those with diabetes, hypertension, or coronary artery disease. The only difference between cases and controls was the presence or absence of AF. We were trying to discover whether there were any LDs that were found exclusively in the cases or exclusively in the controls, which might indicate the existence of epistasis.

We found that there were many significant LDs that were only found either in the cases or in the controls. Many of these LDs crossed chromosomal boundaries. LDs that were

\begin{table}
\centering
\caption{Multilocus Interaction Model by MDR Method}
\begin{tabular}{lll}
\hline
Locus No. and Combination & Cross-Validation & Prediction \\
& Consistency & Error, % \\
\hline
2-locus: M235T/A-20C & 8.9 & 42.36 \\
3-locus*: T174M/M235T/ACE & 10.0* & 37.26* \\
4-locus: T174M/M235T/G-6A/ID & 8.4† & 39.42† \\
5-locus: T174M/M235T/G-6A/G-152A/ID & 4.5 & 40.29 \\
6-locus: T174M/M235T/G-6A/G-217A/ID/AT,R & 3.9 & 42.91 \\
7-locus: T174M/M235T/G-6A/A-20C/G-217A/ID/AT,R & 6.7 & 44.03 \\
\hline
\end{tabular}
\end{table}

\*P<0.001 based on 1000 permutations.
†P=0.01 based on 1000 permutations.

\begin{table}
\centering
\caption{Differences of Pattern of Significant Multilocus LD in Patients With AF and Controls}
\begin{tabular}{lll}
\hline
P* AF & P* Control & Locus Combination \\
\hline
(n=250) & (n=250) & \\
\hline
Locus combination with significant LD in patients with AF but not in controls \\
0.000 & 0.132 & T174M/M235T \\
0.000 & 0.156 & T174M/G-6A \\
0.000 & 0.084 & G-6A/G-152A \\
0.000 & 0.072 & G-6A/G-217A \\
0.000 & 0.092 & G-6A/AT,R \\
0.000 & 0.090 & T174M/M235T/AT,R \\
0.000 & 0.309 & T174M/G-6A/G-152A \\
0.000 & 0.369 & T174M/G-6A/G-217A \\
0.000 & 0.100 & T174M/G-6A/AT,R \\
0.000 & 0.155 & G-6A/G-217A/AT,R \\
0.000 & 0.223 & T174M/G-6A/G-152A/G-217A \\
0.000 & 0.094 & T174M/G-6A/G-152A/AT,R \\
0.000 & 0.283 & T174M/G-6A/G-217A/AT,R \\
0.000 & 0.061 & G-6A/G-217A/ACE/AT,R \\
0.000 & 0.113 & T174M/G-6A/G-152A/G-217A/AT,R \\
\hline
Locus combination with significant LD in controls but not in patients with AF \\
0.057 & 0.000 & A-20C/G-152A/AT,R \\
0.069 & 0.001 & A-20C/ACE/AT,R \\
0.092 & 0.000 & A-20C/G-152A/G-217A/AT,R \\
0.066 & 0.000 & T174M/M235T/G-152A/G-217A/ACE/AT,R \\
0.110 & 0.000 & M235T/A-20C/G-152A/G-217A/ACE/AT,R \\
\hline
\end{tabular}
\end{table}

*P values are corrected by multiple tests (Bonferroni correction).
only found in the cases indicate that interactions between RAS genes may predispose patients to the occurrence of AF, whereas LDs that were only found in the controls may play a protective mechanism against the occurrence of AF.

Study Limitations
There are limitations to our studies. First, the present results only provide evidence of the association between RAS gene variations and AF at the gene level and do not demonstrate the direct mechanism by which RAS causes AF. Second, we did not include patients with lone AF in the present study. However, the cause of lone AF may be primarily an ionic mechanism,13 and the RAS may not play as important a role as in secondary structural AF. Furthermore, given the low numbers of patients with lone AF in clinical practice, the association of RAS gene polymorphisms with secondary AF is more clinically relevant. Third, MDR and multilocus LD models may be difficult to interpret, and the patterns of multilocus genotype combinations of these 2 tests were not consistent. Sorting out the nature of the interactions in multidimensional space to infer function remains an interpretive challenge.

Conclusions
Development of nonfamilial structural AF may be genetically controlled to some extent. Patients who have a specific genetic variation or polymorphism in the RAS genes may be more liable to develop AF when exposed to environmental factors that elevate atrial pressure. This study is in a Chinese population, and its applicability to other ethnic group is uncertain and warrants further study.

Acknowledgments
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