Association of ACE Gene Polymorphism with Genetic Susceptibility to Systemic Lupus Erythematosus in a Chinese Population: A Family-based Association Study

JIANHUA XU, YONG WANG, FAMING PAN, JIM STANKOVICH, DONGQING YE, LI LIAN, KECHUN ZHANG, and CHANGHAI DING

ABSTRACT. Objective. Family-based association analysis was performed to investigate whether the angiotensin-converting enzyme (ACE)-G261T and ACE-A592G polymorphisms are risk factors for systemic lupus erythematosus (SLE) in a Chinese population.

Methods. A total of 119 patients with SLE from 95 nuclear families, aged from 14 to 78 years, who met the American College of Rheumatology 1997 criteria, were recruited, as were 316 family members of these patients. A family-based association study was used to explore the association between ACE gene polymorphisms (ACE-G261T and ACE-A592G) and SLE. The ACE gene polymorphisms were genotyped with restriction fragment length polymorphism-polymerase chain reaction.

Results. Using family-based association tests, the T allele of the ACE-G261T single-nucleotide polymorphism (SNP) was significantly associated with genetic susceptibility to SLE in an additive model (Z = 2.877, p = 0.004), a dominant model (Z = 2.557, p = 0.011), and a recessive model (Z = 2.202, p = 0.028). No association between the ACE-A592G SNP and SLE susceptibility was determined.

Conclusion. The T allele of the ACE-G261T SNP was associated with SLE in this Chinese population.

Key Indexing Terms:
ANGIOTENSIN-CONVERTING ENZYME SINGLE-NUCLEOTIDE POLYMORPHISM SUSCEPTIBILITY SYSTEMIC LUPUS ERYTHEMATOSUS FAMILY-BASED ASSOCIATION TEST

Systemic lupus erythematosus (SLE) is an autoimmune disorder of unknown etiology that is known to occur in genetically susceptible patients influenced by environmental factors. It is likely that the genetics of SLE is complex, with many genes influencing susceptibility. A number of genetic association studies have identified various candidate lupus susceptibility genes. These include members of the HLA family, complement genes, mannose-binding protein, Fcγ receptors, interleukin 10 (IL-10), and recently, angiotensin-converting enzyme (ACE). ACE is expressed in a wide range of tissues including lung, vascular endothelium, kidney, heart, and testes. ACE activates angiotensin I into angiotensin II and inactivates bradykinin via the kallikrein–kininogen system, and plays a major role in the rennin–angiotensin system (RAS). Angiotensin II increases vascular smooth-muscle cell contraction and affects smooth-muscle proliferation, monocyte adhesion, and platelet adhesion and aggregation, being mediated either directly or via various factors such as endothelin, nitric oxide, and others. Angiotensin II is also a potent proinflammatory modulator with the ability to augment and perpetuate immune responses in renal and non-renal tissues. Since immunological abnormalities appear to be directly related to the disease process of SLE, it is reasonable to predict that ACE gene polymorphisms may contribute to SLE susceptibility by affecting the function of the ACE protein or the systemic or local concentrations of ACE, which subsequently alters the immunological response in patients with lupus. However, the role of ACE gene polymorphisms such as insertion/deletions (I/D) in the pathogenesis of SLE remains unresolved. A search for additional ACE gene polymorphisms may be helpful to elucidate the role of ACE in SLE. The aim of our study was, therefore, to determine whether 2 ACE gene polymorphisms, ACE-G261T and ACE-A592G, are associated with SLE in a Chinese population.

MATERIALS AND METHODS

Study participants. A family-based association study was carried out in 119 subjects with SLE (classified according to the American College of
Rheumatology 1997 revised criteria) from 95 nuclear families and 316 other family members (age 37.4 ± 14.6 yrs). They were recruited from the Department of Rheumatology at First Affiliated Hospital, Anhui Medical University. Of the families, 47 (39.5%) had both parents of patients available, 27 (22.7%) had 1 parent available, and 45 (37.8%) had no parent available. The family criteria used for subject selection were as follows: (1) 2 parents and proband; (2) if 1 parent was missing or deceased, at least 1 or 2 normal siblings were recruited. The structures of families with SLE in our study are presented in Table 1. All subjects gave their written informed consent, and the study protocol was approved by the Medical Human Research Ethics Committee at Anhui Medical University.

Markers. Two single-nucleotide polymorphisms (SNP) of ACE gene were selected from the National Center for Biotechnology Information dbSNP database that met the following criteria: (1) the SNP were located in exons; (2) the SNP had high heterozygosity (selective standard: heterozygosity > 5%); and (3) the SNP were nonsynonymous. Of the 2 SNP residing in the ACE gene we selected, rs4303 (G → T transition; Ala → Ser) is located in exon 5 (at amino acid position 261), and rs12709426 (A → G transition; Asp → Gly) is located in exon 12 (at amino acid position 592).

Phlebotomy. Forearm venous blood samples were collected from each subject by venipuncture into 10-ml vacuum tubes containing EDTA and citrate and put on ice. The tubes were centrifuged at 2000 rpm for 10 min, and then the plasma was excluded from the cell pellet by pipetting. All samples were stored at −80°C.

Genotyping of ACE polymorphisms. Genomic DNA was extracted from the cell pellet in whole blood (QIAamp blood kit, Qiagen) and stored at −20°C until the genotype analysis was performed. ACE-G261T polymorphism was genotyped by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) method using the following primers: sense and antisense primers were 5′-TCT ACC TGA ACC TCC ATG CC-3′ and 3′-GGT CCT TAC CCA GCA GAT GA-5′, respectively. The 108-bp PCR amplicon was digested by the Hhal restriction enzyme [total reaction volume 15 µl, including PCR product 10 µl, 10 × buffer 1.5 µl, restriction enzyme 0.4 µl (5 u/10 µl), H2O 3.1 µl], and then was separated by electrophoresis on 2.5% agarose gel. The PCR fragment for the heterozygous ACE-261G allele was digested into 3 fragments of 108, 32, and 76 base pairs. Each reaction mixture contained 20 ng of genome DNA, 0.15 µM of each primer, 0.4 mM dNTPs, and 2.5 units Taq (heat-resistant) DNA polymerase (Takara; Otsu, Shiga, Japan), using a T-gradient thermocycler (Biometra, Göttingen, Germany). The amplification procedure consisted of initial denaturation at 96°C for 3 min, 35 cycles of denaturation at 96°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 90 s, followed by a final extension at 72°C for 7 min until genotype analysis was performed following a standard protocol. PCR was performed in 10 µl volumes.

Statistical analysis. Statistical analysis was performed using the family-based association test (FBAT)10, an extension of the transmission disequilibrium test. The test was performed using dominant, recessive, and additive genetic models. The number of informative families is dependent on the genetic model used, and the statistical power of the FBAT depends on the number of informative families. Single-marker FBAT analysis was used to estimate the single-loci frequencies. Each test counts how often a specific locus is present in informative families with SLE. A positive Z statistic in a single-locus analysis showed that the ACE-261T allele was significantly associated with genetic susceptibility to SLE in the Chinese population. The morphism was significantly associated with genetic susceptibility to SLE in the Chinese population. The results of our study suggest that DNA sequence variation in the ACE gene influences the risk of developing SLE.

Table 1. Genotype distributions of ACE-G261T polymorphism and Hardy-Weinberg expectation test in systemic lupus erythematosus.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Observed, n</th>
<th>Expected, n</th>
<th>Chi-square</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4303</td>
<td>G/G</td>
<td>17</td>
<td>24</td>
<td>3.434</td>
<td>0.180</td>
</tr>
<tr>
<td></td>
<td>G/T</td>
<td>73</td>
<td>59</td>
<td>2.202</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>29</td>
<td>36</td>
<td>2.202</td>
<td>0.028</td>
</tr>
</tbody>
</table>

DISCUSSION

In our study, we found that the ACE-G261T (rs4303) polymorphism was significantly associated with genetic susceptibility to SLE, and demonstrated that the ACE-261T allele was transmitted to affected offspring (p = 0.004). Therefore, the ACE gene may play an important role in influencing susceptibility to SLE in the Chinese population. The results of our study suggest that DNA sequence variation in the ACE gene influences the risk of developing SLE.
have demonstrated either association with the I allele\(^{14-16}\) or no association with either allele\(^{5,7,17-19}\). Results have also varied in terms of the specificity of the association, with some studies demonstrating association with SLE in general\(^{12-15}\), and other associations restricted to specific clinical subgroups, such as lupus nephritis (LN), active LN, or disease activity\(^{11,16,20}\). One study reported the ACE I/D polymorphism was associated with SLE among non-Caucasians and the 23949 (CT)\(_{2/3}\) polymorphism was associated with LN among non-Caucasians\(^{31}\). Another multivariate analysis demonstrated associations between the ACE I/D, ACE (CT)\(_{2/3}\), and Atg C-532T functional polymorphisms and LN among Asians\(^{22}\). In stratified analyses among LN cases according to high versus low glomerular filtration rate (GFR), associations remained significant for the ACE D and (CT)\(_2\) alleles among Asian subjects with low GFR\(^{22}\). Careful review of these previous studies in SLE suggests that much of the discordance may be secondary to heterogeneity, study designs, power, disease state, phenotype definition, and ethnic variability. This also suggests the need to genotype additional ACE gene polymorphisms in order to understand the role of ACE in SLE.

We report for the first time in our study the association of the G261T SNP of the ACE gene with SLE in a Chinese population using a family-based association method. Further studies with larger sample sizes, specific ethnic subgroups, and additional polymorphisms in the region are needed to confirm and extend these results.

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**REFERENCES**