Original Article

Association between ACE polymorphisms and osteoarthritis susceptibility

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Abstract: Objective: The present study was designed to investigate the association of angiotensin-converting enzyme (ACE) rs4343 and rs4362 polymorphisms with the susceptibility to osteoarthritis (OA). Methods: 109 knee OA patients and 114 healthy people were enrolled in the study. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to perform the genotyping for two groups and the linkage disequilibrium and haplotype were analyzed using Haploview software. The differences of genotype and allele frequencies were analyzed by χ² test and Fisher’s exact test. The relationship between ACE polymorphisms and OA susceptibility was represented by odds ratios (ORs) with 95% confidence intervals (95% CIs). Results: The genotypes distributions of ACE rs4343 and rs4362 polymorphisms in control groups were accordance with HWE. ACE rs4343 polymorphism was associated with the significantly increased risk of OA (AG vs. AA: OR=2.41, P=0.003; GG vs. AA: OR=5.35, P=0.015; G vs. A: OR=2.27, P<0.001). Similarly, rs4362 polymorphisms was also a risk factor for OA (CT vs. CC: OR=2.60, P=0.005; TT vs. CC: OR=3.15, P=0.003; T vs. C: OR=1.88, P=0.001). The result of haplotype analysis showed complete linkage disequilibrium in rs 4343 and rs 4362 polymorphisms. The G-T haplotype significantly increased OA susceptibility, but A-C is a protective factor for the occurrence of OA. Conclusion: Significant correlation exists between ACE rs4343 and rs4362 polymorphisms and OA. In haplotype analysis, A-C haplotype may provide protection against OA, and G-T haplotype may be a risk factor for the development of OA.

Keywords: ACE, osteoarthritis, polymorphism, risk

Introduction

Osteoarthritis (OA) is a common disease of joint surgery that can lead to the loss of joint function [1]. The pathological change of OA involves all structures of joint, especially cartilage [2]. OA can cause the arthralgia, malformation and dysfunction of joint, the sclerosis of subchondral bone, joint space narrowing, syndesmophyte formation, synovitis and the contracture or flab of ligament [3, 4]. The worst result makes the patients disabled and affects the quality of life seriously [5, 6]. Understanding the pathological mechanism of OA is the only way to find effective methods for preventing and reducing the occurrence of OA.

Epidemiological studies point out that OA is a complex disease involving combined actions of genetic and environment factors as well as multiple other factors [7, 8]. Age, hormone, and trauma are all a relationship with the occurrence and development of OA [9]. Additionally, OA has been shown in studies to have a strong genetic predisposition and the pathogenesis may be associated with some genetic polymorphisms [10, 11].

Angiotensin-converting enzyme (ACE) is a key enzyme of renin-angiotensin system (RAS), and plays an important part in maintaining the stabilization of water, electrolyte and internal environment in human body [12]. ACE can regulate the physiological function of blood vessel, for example, it can catalytically translate angiotensin I into angiotensin II shrinking blood vessel and secreting aldosterone, and also inactivate vasodilator bradykinin to affect neurotransmitter metabolism [13]. Molecular biology studies have found that a variety of polymorphisms exist on ACE gene. Freire et al. demonstrated that the seventh intron of ACE contains restriction fragment polymorphisms caused by the differences of two sequences [14]. Lu et al. also
has proved that ACE has I/D (rs4340) polymorphism associated with the expression level and activity of ACE protein in human body [15]. McKenzie et al. pointed out that ACE rs4343 polymorphism on exon 17 is highly related to the expression level of ACE [16].

In recent years, the relationship between ACE polymorphisms and diseases has become a hot spot in research field. However, most of studies focus on the pathogenesis of hypertension, diabetes and cardiovascular diseases [17-20]. Poornima et al. have certified that ACE polymorphisms are closely related to type 2 diabetes and gestational diabetes mellitus [21]. Meanwhile, Narne et al. also suggested that the genetic variant of ACE played important roles in coronary artery disease (CAD) and myocardial infarction (MI) [22]. The previous studies, however, choose one single polymorphism (mainly rs4340) as the target, being disadvantageous to comprehensive study. Our study explored two polymorphisms of ACE rs4343 and rs4362 to investigate the association between ACE polymorphisms and OA susceptibility, which provides theoretical basis for further exploration of OA pathogenesis and the early prevention.

**Materials and methods**

**Subjects**

All subjects including 109 knee OA patients and 114 healthy people were recruited from Xi'an Honghui Hospital. The OA patients were confirmed by clinical examination and radiographic inspection. Patients were excluded if they suffered from other kinds of arthritis such as traumatic arthritis (TA), infective arthritis and skeletal dysplasia, or had tumor history. According to the Kellgren/Lawrence standard, patients with a score of 2 or more would be enrolled in the case group. The healthy controls without the history of tumors, immunopathy and osteoarthritis disease were collected from the physical examination center of the same hospital. They were frequency-matched with cases by age, gender and body mass index (BMI). To ensure the accuracy of the study, all subjects belonged to Chinese Han population for a long time and were not related by blood. Our research obtained the approval from the Ethics Committee and written informed consent from each participant.

**Collection of clinical data and sample from study subjects**

The clinical information of all subjects in two groups were collected, including age, gender, onset age, weight, stature and body mass index (BMI= Weight (kg)/Height²), especially the history of relevant diseases, such as hypertension, diabetes and thyroid dysfunction.

5 ml fasting peripheral venous blood from every subject was collected in EDTA anticoagulant tubes. Genome DNA was extracted with DNA extraction kit according to manufacturer instructions. DNA samples were conducted the test of quality and concentration with ultraviolet spectrophotometer, and preserved at -20°C refrigerator.

**Genotyping of ACE polymorphisms**

PCR primers of ACE rs4343 and rs4362 polymorphisms were designed using Primer premier 5.0 software and synthesized by Shanghai Sangon Biological Engineering Technology Service Co., LTD. The PCR reactions system was a volume of 25 μL solution, including 1 μL DNA template substrates, 12.5 μL Master Mix (2×), each 0.5 μL forward and reverse primers and was added with aseptic dd H₂O to 25 μL. PCR conditions were as follows: initial denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 50 s, extending at 72°C for 30 s and final extension at 40°C for 5 min. PCR products were digested by restriction enzyme Hal III and MboI, 2% agarose gel electrophoresis was conducted to separate these restriction fragments.

**Statistical analysis**

Experimental data were shown as X±SD. The representativeness of genotypes in the control group was estimated by Hardy-Weinberg equilibrium (HWE). The differences of genotype and allele distributions were analyzed by χ² test and Fisher’s exact test, and represented by odds ratios (ORs) and 95% confidence intervals (CIs). Statistical analysis was conducted by SPSS 18.0 software. Haplovieview software was used to calculate the linkage disequilibrium and haplotype composition among SNPs with statistical significance when P<0.05.
Results

Comparison of clinical data of the subjects

The clinical data of all subjects are shown in Table 1. The average BMI of 109 cases (47 males, 62 females) was 26.52±3.74 kg/m², their median age was 54.25±9.75 while the average onset age was 42.54±7.28. The BMI of 114 controls (51 males, 63 females) was 24.27±2.78 kg/m². Their average age was 52.13±8.59. No statistically significant differences existed in age, sex and BMI between two groups (P>0.05). However, significant differences were exposed in such aspects as hypertension, diabetes and thyroid dysfunction (P<0.05) through the investigation of participants' disease history. Data indicated that the onset of OA had a relationship with hypertension, diabetes and immune organ diseases.

HWE test

The HWE test showed the genotype distributions of ACE rs4343 and rs4362 polymorphisms were consistent with the law of genetic equilibrium in control group (rs4343: $\chi^2=0.109$, $P=0.742$; rs4362: $\chi^2=2.521$, $P=0.112$), which indicated that our subjects were randomly selected and had demographic representativeness.

Correlation analysis of ACE polymorphisms and OA risk

The genotype and allele distributions of ACE rs4343 and rs4362 polymorphisms in two groups are shown in Tables 2 and 3. The genotype and allele distributions of rs4343 and rs4362 in two groups were significantly different ($P<0.05$) indicating that G allele at rs4343 could increase the risk of OA (AG vs. AA: OR=2.41, 95% CI=1.37-4.23; GG vs. AA: OR=5.35, 95% CI=1.40-20.4; G vs. A: OR=2.27, 95% CI=1.46-3.54) and T allele at rs4362 might be the susceptible factor for OA (CT vs. CC: OR=2.60, 95% CI=1.37-4.93; TT vs. CC: OR=3.15, 95% CI=1.49-6.68; T vs. C: OR=1.88, 95% CI=1.29-2.74). Compared with T allele in rs4362, G in rs4343 was more likely to be the susceptible allele to OA.

Linkage disequilibrium and haplotype analysis

Linkage disequilibrium analysis showed that complete linkage disequilibrium existed between ACE rs4343 and rs4362 ($D'=1.0$, $r^2=0.832$). Further analysis of the frequencies difference of established haplotypes between

Table 1. Comparison of clinical information of the subjects

<table>
<thead>
<tr>
<th>Clinic information</th>
<th>Case (n=109)</th>
<th>Control (n=114)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>54.25±9.75</td>
<td>52.13±8.59</td>
<td>0.765</td>
</tr>
<tr>
<td>Sex (male: female)</td>
<td>47:62</td>
<td>51:63</td>
<td>0.893</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.52±3.74</td>
<td>24.27±2.78</td>
<td>0.698</td>
</tr>
<tr>
<td>Onset age</td>
<td>42.54±7.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension [n (%)]</td>
<td>41 (37.61)</td>
<td>11 (9.65)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes [n (%) ]</td>
<td>32 (29.36)</td>
<td>9 (7.89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thyroid dysfunction [n (%)]</td>
<td>35 (32.11)</td>
<td>18 (15.79)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table 2. The genotype and allele distribution comparison of ACE gene rs4343 polymorphism of the subjects

<table>
<thead>
<tr>
<th>rs4343</th>
<th>Case (n=109, %)</th>
<th>Control (n=114, %)</th>
<th>$\chi^2$</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>48 (44.0)</td>
<td>77 (67.6)</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>AG</td>
<td>51 (46.8)</td>
<td>34 (29.8)</td>
<td>9.47</td>
<td>0.003</td>
<td>2.41 (1.37-4.23)</td>
</tr>
<tr>
<td>GG</td>
<td>10 (9.2)</td>
<td>3 (2.6)</td>
<td>7.17</td>
<td>0.015</td>
<td>5.35 (1.40-20.4)</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>147 (67.4)</td>
<td>188 (82.5)</td>
<td>13.5</td>
<td>&lt;0.001</td>
<td>2.27 (1.46-3.54)</td>
</tr>
<tr>
<td>G</td>
<td>71 (32.6)</td>
<td>40 (17.5)</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Table 3. The genotype and allele distribution comparison of ACE gene rs4362 polymorphism of the subjects

<table>
<thead>
<tr>
<th>rs4362</th>
<th>Case (n=109, %)</th>
<th>Control (n=114, %)</th>
<th>$\chi^2$</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>22 (20.2)</td>
<td>47 (41.2)</td>
<td>-</td>
<td>-</td>
<td>1.000</td>
</tr>
<tr>
<td>CT</td>
<td>56 (51.4)</td>
<td>46 (40.4)</td>
<td>8.79</td>
<td>0.005</td>
<td>2.60 (1.37-4.93)</td>
</tr>
<tr>
<td>TT</td>
<td>31 (28.4)</td>
<td>21 (18.4)</td>
<td>9.26</td>
<td>0.003</td>
<td>3.15 (1.49-6.68)</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>100 (45.9)</td>
<td>140 (61.4)</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
</tr>
<tr>
<td>T</td>
<td>118 (54.1)</td>
<td>88 (38.6)</td>
<td>10.8</td>
<td>0.001</td>
<td>1.88 (1.29-2.74)</td>
</tr>
</tbody>
</table>
two groups manifested that there were 3 haplotypes formed by rs4343 and rs4362: A-C, A-T and G-T. The haplotype frequencies difference of A-C and G-T between two groups had statistical significance \((P<0.05)\). The result from the study indicated that \(\text{ACE}\) haplotype A-C might offer protection against OA \((\text{OR}=0.533, \ 95\% \ CI=0.365-0.777)\), and that G-T haplotype might increase the risk of OA \((\text{OR}=2.270, \ 95\% \ CI=1.457-3.537)\) (Table 4).

### Discussion

Osteoarthritis (OA) is a degenerative joint disease caused by a variety of factors, and there are tens of millions of OA patients waiting for cure \([23]\). However, the pathogenesis of OA has not entirely clear yet. Studies in recent years have proved that inflammation plays an important role for the occurrence of OA and \(\text{ACE}\) is closely associated with rheumatic and autoimmune diseases \([24-26]\). Therefore, the present research explored the relationship of \(\text{ACE}\) rs4343 and rs4362 polymorphisms with the susceptibility to OA to provide certain theoretical basis for prevention and treatment.

\(\text{ACE}\) (about 21 kb) is located on chromosome 17 q23, containing 26 exons and 25 introns \([27]\). Although \(\text{ACE}\) has many polymorphisms, most studies focus on the insert and/or deletion (I/D) fragment polymorphisms of the 16th intron widely. Two new SNPs of \(\text{ACE}\) were explored in the present study. Rs4343 polymorphism in \(\text{ACE}\) exon 17 has been certified by previous studies to have some correlation with essential hypertension, heart disease, nephropathy and Alzheimer disease \([28-30]\), but there was no report on its association with osteoarthritis. Additionally, rs4362 polymorphism of \(\text{ACE}\) exon 24 had not been reported its relationship with osteoarthritis either.

Our study showed that the differences between knee osteoarthritis patients and healthy controls in disease history including hypertension, diabetes and immune organ diseases had statistical significance \((P<0.05)\), indicating that the development of OA was related to hypertension, diabetes and immune organ function. The genotype and allele distributions of rs4343 and rs4362 polymorphisms demonstrated significant differences between cases and controls \((P<0.05)\). The rs4343 G allele could increase OA risk, similarly, the rs4362 T allele might be the susceptible factor for OA. The further haplotype analysis of rs4343 and rs4362 polymorphisms suggested that A-C haplotype might have a protective effect against the incidence of OA, but G-T haplotype might increase the risk of OA.

The rs4343 and rs4362 polymorphisms are located in exon region of \(\text{ACE}\), and both of them are synonymous mutations. The replacement of bases does not change amino acid composition in \(\text{ACE}\). However, McKenzie et al. demonstrated that rs4343 polymorphism could affect the expression level of \(\text{ACE}\) in serum or tissues and G allele could lead to a significant change in \(\text{ACE}\) expression level. Therefore, it was inferred that \(\text{ACE}\) rs4343 and rs4362 polymorphisms might affect the susceptibility to OA by influencing the expression level of \(\text{ACE}\) in serum or tissues. Besides, our research combined two polymorphisms of rs4343 and rs4362 in \(\text{ACE}\) gene to evaluate their relationship with the progress and occurrence of OA susceptibility, so it was more convincing than one single polymorphism. The polymorphisms of \(\text{ACE}\) rs4343 and rs4362 may participate jointly in the pathophysiology of OA, but the exact mechanism needs to be further explored.

### Disclosure of conflict of interest

None.

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