Association study of Angiotensin I converting enzyme (ACE) gene polymorphisms with an osteonecrosis of femoral head in Korean population

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INTRODUCTION

Osteonecrosis of the femoral head (ONFH) is a bone disorder that causes the temporary or permanent loss of blood flow to the bones and usually affects middle aged men between 30 and 50 years of age. Although the factors developing of ONFH are variable, the exact pathogenesis of non-traumatic ONFH is still unknown. Non-traumatic ONFH has been associated with corticosteroid usage, alcoholism, infections, marrow infiltrating diseases, and coagulation defects. All of these risk factors are closely related with direct and indirect injury to the vascular supply to the bone.

ACE is an enzyme that catalyzes the conversion of angiotensin I into angiotensin II. ACE has been known to play a key role in the renin-angiotensin system that regulates blood pressure. ACE II was shown to regulate VEGF-induced angiogenic activity and play a key role in the development of vascular proliferation. The treatment of ACE inhibitors was associated with a decreased risk for bone fracture and higher bone mineral density (BMD) in the femoral neck, hip and lumbar spine 207,208. Recent studies showed that Angiotensin II accelerated osteoporosis by activating osteoclast. Moreover, Angiotensin I and II have been known to stimulate osteoclastic bone resorption. Therefore, ACE might influence a number of physiological processes including bone metabolism.

These results have suggested that ACE was an important contributor to the vascular remodeling processes including the bone. Thus, we performed the association analysis of ACE polymorphisms, to assess genetic effect on risk of ONFH.

METHODS

Subjects

A total of 443 (366 men, 77 women; age: 49.7±13.3) unrelated patients with ONFH and 273 (206 men, 67 women; age: 52.1±10.6) unrelated control subjects were consecutively enrolled at the Kyungpook National University Hospital (Daegu, Korea) from 2002 to 2006. According to etiological factors, patients were subgrouped into idiopathic (186 cases), steroid-induced (59 cases) and alcohol-induced (215 cases) osteonecrosis groups. The diagnosis was made using anteroposterior and lateral pelvic radiographs and magnetic resonance images. Steroid-induced ON was defined by a history of taking large dosages of steroids due to nephritic syndrome, organ transplantation, systemic lupus erythematosus, and rheumatic arthritis. Alcohol-induced ON was diagnosed by a history of pure ethanol consumption more than 400 ml of alcohol per week. A control subjects were recruited from spouses of the patients and the general population. All individuals gave informed consent for study participation and the study was approved by the Institutional Review Board.

Genotyping

The genotyping was performed using the Affymetrix® Targeted Genotyping (TG) 3K chip array. A TG chip using molecular inversion probe (MIP) technology with Gene chip universal microarrays provides a method that is capable of analyzing thousands of variants in a single reaction. The basic concept of MIP technology has been described previously. The genotyping reactions were carried out using the standard protocols recommended by the manufacturer (Affymetrix). The arrays were scanned with the GeneChip Scanner 3000 7G, and the images were analyzed using GCOS software (Affymetrix). Finally, the TG analysis software measures the data quality and generates genotypes for arrays which have met a specific set of quality control criteria.

Statistical analysis

Statistical analysis was based on the frequency of alleles and genotypes between the control and case in ONFH. Hardy-Weinberg equilibrium was tested for each SNP using the χ2 test. Logistic regression models were used for calculating adjusted odds ratios (OR) and their 95% confidence interval (CI). Four SNPs in the exon and five SNPs in the intron were genotyped. The genotype distribution of nine polymorphisms did not deviate from the Hardy-Weinberg equilibrium in our sample set. We found the significant association for two SNPs (rs4309 and rs4344) of ACE in the dominant model (P=0.0147, 0.0367, OR 1.50, 1.42, respectively). A SNP (rs461142) was associated with the increased risk of ONFH in all alternative analysis models except for the recessive model (P=0.0044-0.0139, OR 1.32-1.63).

For further analysis, we classified the patients on the basis of pathological etiology into three subgroups (alcohol, idiopathic, and steroid-induced ONFH subgroups). We found the most significant association of five SNPs (rs4295, rs4309, rs4344, rs4362 and rs461142) of ACE with steroid induced ONFH (P<0.005, OR >3).

Table. Association analysis for ACE gene among ONFH patients and controls.

<table>
<thead>
<tr>
<th>rs ID</th>
<th>Genotype</th>
<th>Co-dominants</th>
<th>Dominant</th>
<th>Recessive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR(95% CI)</td>
<td>OR(95% CI)</td>
<td>OR(95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>rs4309</td>
<td>T&gt;C</td>
<td>1.24(0.99-1.54)</td>
<td>1.50(1.08-2.08)</td>
<td>1.09(0.73-1.62)</td>
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<tr>
<td>rs4344</td>
<td>A&gt;G</td>
<td>1.17(0.93-1.47)</td>
<td>1.42(1.02-1.96)</td>
<td>0.98(0.65-1.48)</td>
</tr>
<tr>
<td>rs461142</td>
<td>C&gt;T</td>
<td>1.34(1.07-1.68)</td>
<td>1.63(1.16-2.28)</td>
<td>1.26(0.84-1.87)</td>
</tr>
</tbody>
</table>

CONCLUSION

In conclusion, we report genetic association study of ACE gene in ONFH patients and normal control. Our results suggested that ACE gene polymorphisms are related to nontraumatic ONFH and may contribute to identifying genetic susceptibility factors of ONFH in Korean population.

REFERENCES


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