Association study of Vascular endothelial growth factor C (VEGFC) 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 disorders that cause 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.

Vascular endothelial growth factor (VEGF), an essential regulator for angiogenesis, plays a role in vascular endothelial cell migration, proliferation and permeability. It is highly expressed in the edematous zone of the ON adjacent to the necrotic area indicating that it might be an important factor for bone tissue repair. Many studies showed that it enhanced angiogenesis and improved the blood flow in necrotic bone. Previous studies have suggested that the dysregulation of angiogenesis and repairing process may affect the progress of ONFH. VEGFC, a member of the VEGF family, binds to KDR and VEGFR3. VEGFC is active in angiogenesis and endothelial cell growth, and can also affect the permeability of blood vessel. VEGFC is an important regulator of lymphangiogenesis and contributes to the development and progression of angiogenic diseases.

Thus, we performed the association analysis of VEGFC 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 (186 cases) osteonecrosis groups. The diagnosis was made using anteroposterior and lateral pelvic radiographs and magnetic resonance imaging. Steroid-induced ON was defined by a history of taking large doses 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), corresponding P-values for SNP sites. All analyses were two-tailed and a p-value < 0.05 was considered statistically significant. Statistics were performed using SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS
Five SNPs (rs2333496, rs1485766, rs3775203, rs3775202 and rs3775194) were genotyped in the intron. The distribution of the observed genotypes did not deviate from the Hardy-Weinberg (P>0.05). Two SNPs (rs1485766 and rs3775203) of VEGFC were associated with an increased risk of ONFH in all alternative analysis models except for the recessive model (P=0.0042-0.0107, OR 1.33-1.67). On the other hand, rs2333496 showed the protective effect of ONFH in the recessive model (P=0.0087, OR 0.55).

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 for several SNPs of VEGFC in alcohol and idiopathic induced subgroup. Two SNPs (rs1485766 and rs3775203) of VEGFC showed their association with both alcohol and idiopathic ONFH. These results suggested that rs1485766 and rs3775203 had an association with the risk of developing ONFH.

Table. Association of VEGFC gene between the ONFH patients and controls in idiopathic subgroup.

<table>
<thead>
<tr>
<th>SNP rs ID</th>
<th>Co-dominant OR(95% CI)</th>
<th>Dominant OR(95% CI)</th>
<th>Recessive OR(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2333496</td>
<td>0.70(0.53-0.93)</td>
<td>0.78(0.53-1.15)</td>
<td>0.36(0.18-0.70)</td>
</tr>
<tr>
<td>rs1485766</td>
<td>1.59(1.20-2.1)</td>
<td>2.26(1.39-3.66)</td>
<td>1.55(1.01-2.39)</td>
</tr>
<tr>
<td>rs3775203</td>
<td>1.38(1.05-1.80)</td>
<td>1.53(0.99-2.38)</td>
<td>1.54(1.00-2.39)</td>
</tr>
<tr>
<td>rs3775202</td>
<td>1.23(0.94-1.61)</td>
<td>1.09(0.73-1.62)</td>
<td>1.77(1.09-2.87)</td>
</tr>
</tbody>
</table>

CONCLUSION
In conclusion, we report genetic association study of VEGFC gene in ONFH patients and normal control. Our results suggested that VEGFC gene polymorphisms are related to nontraumatic ONFH and may contribute to identifying genetic susceptibility factors of ONFH in Korean population. Defining the genetic susceptibility factors for developing ONFH may provide more effective therapeutic implications and early diagnosis for disease.

REFERENCES

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