Association analysis between polymorphisms of the Coagulation factor V gene and the risk of osteonecrosis of femoral head in Korean population

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INTRODUCTION

Osteonecrosis (ON) of the femoral head (ONFH), a debilitating bone disease characterized by cellular death in the bone tissue, can lead to collapse of the architectural bony structure and joint function loss. Although many conditions, such as steroids, alcoholism, coagulation defects, storage diseases, marrow-infiltrating diseases, and some autoimmune diseases, can increase the risk of ONFH, the pathogenesis has not been completely elucidated. Many theories regarding pathogenesis, including intravascular coagulation, apoptosis of the osteoblasts and osteocytes, and fatty necrosis of the osteocytes, have been suggested. Among them, a vascular hypothesis is considered to be the most persuasive, which implicates a decrease in the local femoral head blood flow in ONFH pathogenesis.

In support of this hypothesis, genetic mutations related to coagulation abnormalities, such as factor V Leiden mutation (F5 Leiden, G1691A, Arg506Gln) and 5, 10-methylene-tetrahydrofolate reductase (MTHFR) gene polymorphism (C677T, A1222Val), have been examined to assess the role of genetics in ONFH risk. Three out of four studies investigating the role of the factor V Leiden mutation showed a positive correlation with ONFH in Caucasians. However, Lee et al. reported that there is no relationship between ONFH and increased thrombosis or impaired fibrinolysis in Korean patients with ONFH. Moreover, other studies have reported the absence of factor V (F5) Leiden and 20210A mutations in Koreans. It may be that there are geographic and ethnic differences in the prevalence of these mutations.

Although F5 Leiden mutation is not found in the Korean population, the F5 gene is thought to play an important role in ONFH. Therefore, we performed extensive screening of the F5 gene by direct sequencing to identify the polymorphisms and mutations, and examined genetic association with ONFH risk in a Korean population.

METHODS

Subjects

Patients with ONFH (423 total, 342 men, 81 women; age: 47.0 ± 1.40 years) and unrelated control subjects (348 total, 298 men, 50 women; age: 51.9 ± 10.7 years) were investigated. All individuals were consecutively enrolled at Kyungpook National University Hospital (Daegu, Korea) from 2002 to 2006 and provided informed consent. The study was approved by the Institutional Review Board of Kyungpook National University Hospital. Patients were diagnosed using anteroposterior and lateral pelvic radiographs and magnetic resonance images. Patients with available genotype data were subgrouped according to etiological factors into alcohol-induced (206 cases), steroid-induced (77 cases), and idiopathic (140 cases) ON groups. Controls were defined by a lack of hip pain and by the absence of any lesion with a sclerotic margin or subchondral collapse consistent with ONFH in anteroposterior and frog lateral pelvic radiographs. Relatives of the patients were also excluded from the control group.

Sequence analysis

DNA sequencing was carried out using the promoter and all coding exons and intron-exon boundaries of the HIF1a gene in 24 Korean DNA samples. Reactions were sequenced using DYEnamic ET dye terminator cycle sequencing kit (Amersham biosciences)

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes of each individual using a FlexiGene DNA Kit. The genotype was determined by a TaqMan fluorogenic 5'-nuclease assay using predesigned TaqMan Probes. All reaction was carried out following the manufacturer’s protocol. Genotyping quality control was performed on 10% of the samples by checking duplicates (rate of concordance in duplicates > 99%).

Statistical analysis

Statistical significance was determined by the P-values obtained from the logistical regression analysis, controlling for age and sex as cova variables with three alternative models (codominant, dominant and recessive e). To assess the risk of phenotypes, the odds ratios (ORs) and 95% confidence intervals (CIs), were also estimated using a logistic regression procedure. A p-value of <0.05 was considered to be statistically significant. Statistical analyses were performed by using SAS 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

Sequence analysis

To study a possible genetic effect of the F5 gene on susceptibility to ONFH, we directly sequenced the F5 gene (promoter, 25 exons and boundaries) in 24 Korean individuals and identified 16 known sequence variants (Fig. 1). The factor V Leiden mutation (G1691A) was not found in our study agreement with previous report. According to LDs and allele frequencies, six polymorphisms were selected and genotyped in ONFH (n=423) and controls (n=348). Comparison of ONFH and control subjects using logistic regression models revealed no statistically significant differences in the frequencies of the F5 polymorphisms and haplotypes. Further analysis stratified by etiology (idiopathic, steroid and alcohol) also showed no association.

CONCLUSION

These findings suggest that F5 polymorphisms do not play a significant role in the susceptibility to ONFH in the Korean population.

REFERENCES


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