Lack of Association of MTHFR Gene Polymorphisms with the Risk of Osteonecrosis of the Femoral Head in a Korean Population

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Some studies have suggested that coagulation disorders may be implicated in osteonecrosis of the femoral head (ONFH). The C677T polymorphism of the 5, 10-methylenetetrahydrofolate reductase (MTHFR) gene has been postulated to be a genetic risk factor for venous thromboembolism and osteonecrosis in Caucasians, but this relationship has not been established in other populations. In this study, we conducted case-control analysis of whether MTHFR polymorphisms are associated with ONFH in Korean patients. Fifteen single nucleotide polymorphisms (SNPs) were selected and genotyped in 443 ONFH patients and 273 control subjects using the TaqMan 5' allelic discrimination assay. Comparison of ONFH and control subjects using logistic regression models revealed no statistically significant differences in the frequencies of the MTHFR polymorphisms and haplotypes. Further analysis stratified by etiology also showed no association. These results suggest that MTHFR polymorphisms play no significant role in susceptibility to ONFH in the Korean population.

INTRODUCTION

Osteonecrosis (ON) of the femoral head (ONFH) is a debilitating bone disease characterized by necrosis of the bone tissue, resulting in the collapse of the joint cartilage and femoral head and a subsequent loss of joint function (Assouline-Dayan et al., 2002). Although the pathophysiology of ONFH has not been fully elucidated, several possible causes have been suggested, including thrombophilia and hypofibrinolysis (Glueck et al., 1997), microvessel destruction (Matsui et al., 1992), fat embolism due to altered lipid metabolism, and increased bone marrow pressure with fat cell enlargement (Kawai et al., 1985; Wang et al., 1997).

Recently, an increased tendency for intravascular coagulation has been proposed as a pathogenic mechanism of ONFH (Glueck et al., 1997; Jones, 1992; Zalavras et al., 2004). In support of this hypothesis, protein C and S deficiency, elevated levels of lipoprotein-a, the factor V Leiden mutation, and the prothrombin G20210A mutation have all been identified as genetic risk factors for hypercoagulability and ON (Bjorkman et al., 2004; Wermes et al., 1999; Zalavras et al., 2002b). Hyperhomocysteinemia has also been identified as an independent risk factor for thrombotic events and osteonecrosis (Glueck et al., 2001). Homocysteine promotes the formation of thromboembolic lesions by damaging vascular endothelial cells and enhancing coagulation activity on vascular walls (Lentz et al., 2002). The C677T (alanine to valine) polymorphism in the gene encoding 5, 10-methylenetetrahydrofolate reductase (MTHFR), an enzyme that plays a role in the remethylation of homocysteine, has been identified as a common cause of MTHFR enzyme deficiency (Kang et al., 1991) and has also been postulated to be a genetic risk factor for osteonecrosis (Glueck et al., 2001).

Almost all of the epidemiological evidence supporting associations between genetic polymorphisms and ON has been observed in Caucasian populations. However, there are obvious racial differences in the prevalence of these polymorphisms, and information on their relationship with ON is limited and inconclusive (Hessner et al., 1999; Hsu et al., 2001; Lu et al., 2002). Therefore, the purpose of this study was to investigate whether genetic variants of the MTHFR gene, including the C677T polymorphism, are associated with ONFH in a Korean population.

MATERIALS AND METHODS

Subjects

This study was approved by the Institutional Review Board of Kyungpook National University Hospital, and all individuals participating in the study gave their informed consent. A total of 443 unrelated patients with ONFH (366 men, 77 women; mean age: 49.7 ± 13.3) and 273 control subjects (206 men, 67 women; mean age: 52.1 ± 10.6) were consecutively enrolled at the Kyungpook National University Hospital (Korea) from 2002 to 2006. Patients were diagnosed and subgrouped by criteria that have been described in a previous study (Kim et al., 2002). Moreover, the diagnosis of ONFH was confirmed by CT/MRI. Patients with history of surgery, trauma, or use of corticosteroids were excluded from the study. The control group consisted of patients who were admitted during the same period for orthopedic indications and were matched with the ONFH group for age and sex. Subjects in both groups were of Korean descent.

Methods

Genotyping was performed using the TaqMan 5' allelic discrimination assay (Applied Biosystems, Foster City, CA). The following single nucleotide polymorphisms (SNPs) were selected for analysis: C677T (MTHFR), G663A (fibrinogen), G20210A (prothrombin), A1298C (factor V Leiden), and G1433T (protein C).

Data Analysis

Statistical analysis was performed using chi-square tests and logistic regression models. The statistical significance level was set at 0.05.
The absolute value of Lewontin’s $D'$ (LD) disequilibrium (LD) between loci was measured by using the estimated using a logistic regression procedure. The linkage ratios (ORs) and 95% confidence intervals (CIs), were also covariates with three alternative models (codominant, dominant, logistical regression analyses, controlling for age and sex as significance was determined by the $p$-map.org/index.html.en), a total of 15 single nucleotide poly-


al., 2008). Briefly, patients were subgrouped according to eti-

ological factors, into alcohol-induced (206 patients), steroid-

induced (56 patients), and idiopathic (181 patients) osteonecro-

sis groups. Eleven patients were excluded from the study on

the basis of a demonstrable history of femur fracture (9 pa-

tients) or multiple myeloma (2 patients). Control subjects were

defined by a lack of hip pain and the absence of any lesions

with a sclerotic margin or subchondral collapse consistent with

ONFH in anteroposterior and frog-leg lateral pelvic radiographs.

All persons related to patients were excluded from the control

group. The clinical characteristics of controls and patients are

summarized in Table 1.

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes

collected from each study participant using the FlexiGene

DNA Kit (Qiagen, USA). Using public databases (dbSNP;


map.org/index.html.en), a total of 15 single nucleotide poly-

morphism (SNP) sites on the MTHFR gene were selected on

the basis of their location, allele frequencies, and disease

relevance. Genotypes were determined using a TaqMan™

fluorogenic 5’-nuclease assay with predesigned TaqMan

Probes (Applied Biosystems, USA). All reactions were car-

ried out following the manufacturer’s protocol. Detailed pro-

cedures regarding the PCR reaction and Taqman assay have

been described previously (Lee et al., 2009). Primer Express

(Applied Biosystems) was used to design both the PCR prim-

ers and the MGB TaqMan probes. One allelic probe was la-

beled with the FAM dye, and the other labeled with fluores-

cent VIC dye. The fluorescence data files from each plate

were collected and analyzed using automated allele-calling

software (SDS 2.2, Applied Biosystems). Genotyping quality

control was performed on 10% of the samples by conducting

duplicate analyses. The rate of concordance between duplica-

ted samples was greater than 99%.

Statistical analyses

For subsequent analysis, SNP sites were required to meet the

minimum criteria of a call rate (CR) > 95.0, a minor allele

frequency (MAF) > 0.05, and Hardy-Weinberg equilibrium (HWE)

> 0.05. Significant deviations from HWE in the genotype

frequency of each SNP were evaluated using the $\chi^2$ test. Statisti-

cal significance was determined by the $P$ values obtained from

logistical regression analyses, controlling for age and sex as

covariates with three alternative models (codominant, dominant,

and recessive). To assess the risk of phenotypes, the odds

ratios (ORs) and 95% confidence intervals (CIs), were also

estimated using a logistic regression procedure. The linkage

disequilibrium (LD) between loci was measured by using the

absolute value of Lewontin’s $D'$ ($|D'|$) (Hedrick, 1987). Hap-

loview 3.32 (http://www.broad.mit.edu/mpg/haplovlew/), which

uses an accelerated expectation-maximization algorithm for

haplotype analysis (Barrett et al., 2005), was used to estimate

haplotype structures and their frequencies within LD blocks

from the genotype data. Fisher’s exact test or $\chi^2$ test was ap-

plied to compare the frequency of discrete variables between

controls and patients. Continuous variables were compared by

Student’s $t$-test or ANOVA. All analyses were two-tailed, and $P$

values < 0.05 were considered to be statistically significant.

Power calculation was performed using the Genetic Power

Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/) (Purcell

et al., 2003). Power was estimated with an $\alpha$ of 0.05, assuming

the disease prevalence to be 1% and the risk allele frequencies

to be the values observed in control samples.

RESULTS

In order to investigate the association between MTHFR gene

polymorphisms and ONFH, fifteen polymorphic sites were

genotyped in 443 ONFH patients and 273 control subjects.

Among the 15 SNPs genotyped, six SNP sites did not fulfill our

criteria of a CR > 95.0, MAF > 0.05, and HWE > 0.05 (Table 2).

The $P$ values of each polymorphism that met the criteria were

compared between ONFH patients and controls using logistic

analysis. As shown in Table 3, there were no significant differ-

ences in allele and genotype frequencies between the ONFH

patients and control subjects. When patients were subdivided

by etiology and an association analysis was conducted, none of

the polymorphisms were associated with the risk of ONFH in

any subgroup (data not shown).

Haplotype blocks were constructed using LD coefficients ($D'$)
between all SNP pairs, and two blocks were identified (Fig.

1A). Based on LD coefficients, haplotypes were reconstructed

(Fig. 1B), and haplotype frequencies were compared between

normal controls and ONFH patients. As shown in Table 4, no

significant differences were observed between patients and

controls. Power analysis indicated that more than 80% power in

detecting an association with ONFH was obtained when the
genotype relative risk (GRR) was set at 1.35-1.63 under a

multiplicative model of inheritance.

DISCUSSION

A number of genetic association studies have been conducted to

link specific genes to the pathogenesis of ON. The majority of

these studies have concentrated on gene polymorphisms affect-
ing the coagulation and fibrinolytic systems (Ferrari et al., 2002;

Zalavras et al., 2002a; 2002b). Several studies have reported

that the factor V Leiden mutation (G1691A, Arg506Gln) in-

creases the risk of primary ON (Bjorkman et al., 2004; 2005;

Zalavras et al., 2004), but other studies have failed to observe

Table 1. Clinical profiles of the ONFH patients and control subjects in this study

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 273)</th>
<th>Total (n = 443)</th>
<th>Idiopathic (n = 181)</th>
<th>Alcohol-Induced (n = 206)</th>
<th>Steroid-induced (n = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)*</td>
<td>52.1 ± 10.6</td>
<td>49.7 ± 13.3</td>
<td>49.3 ± 14.2</td>
<td>52.0 ± 11.9</td>
<td>42.5 ± 13.2</td>
</tr>
<tr>
<td>Sex (male/female)*</td>
<td>206/67</td>
<td>366/77</td>
<td>131/50</td>
<td>201/5</td>
<td>34/22</td>
</tr>
<tr>
<td>Affected (Uni/Bilateral)</td>
<td>187/256</td>
<td>89/92</td>
<td>77/129</td>
<td>21/35</td>
<td>21/35</td>
</tr>
<tr>
<td>BMI (kg/m²) (mean ± SD)</td>
<td>23.4 ± 3.01</td>
<td>23.2 ± 2.81</td>
<td>23.2 ± 2.70</td>
<td>23.3 ± 2.85</td>
<td>22.6 ± 3.04</td>
</tr>
</tbody>
</table>

*P < 0.05, for differences between patients and controls

SD, standard deviation