Growth Differentiation Factor 5 (GDF5) Core Promoter Polymorphism Is Not Associated with Susceptibility to Osteoarthritis of the Knee in the Korean Population

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Osteoarthritis (OA), the most common form of arthritis, is characterized by degeneration of articular cartilage.1 The cause of OA is multi-factorial, and aging, hormonal, environmental and genetic factors are among the major risk factors associated with its onset and development.2 Current concepts of OA suggest that it is caused by an imbalance of anabolic and catabolic processes of cartilage in response to mechanical stress with participation by inflammatory mediators.3 Recent research has also shown that genetic factors contribute substantially to the etiology of OA.4,5 These two considerations contribute to the interest of a recent report showing that growth differentiation factor 5 (GDF5) is a susceptibility factor for OA.6

GDF5 is a member of the transforming growth factor-β superfamily and plays a crucial role in the morphogenesis of tendon, ligament, and bone.7 GDF5 mutations have been implicated in several skeletal development disorders, such as various forms of chondrodysplasia, synphalangism, and type C brachydactyly.8 In addition, transgenic mouse studies suggest that GDF5 promotes differentiation of chondrocytes, causing hypertrophy, and enhances commitment of mesenchymal cells to the chondrocyte lineage.9 GDF5 is present in both normal and osteoarthritic articular cartilage, and responsiveness to GDF5 is preserved in
osteoarthritic chondrocytes. These findings indicate that decreased GDF5 expression may lead to OA susceptibility. A functional single nucleotide polymorphism (SNP) in the 5′ untranslated region (UTR) of GDF5 (+104T/C; rs143383) influences transcriptional activity in the GDF5 gene core promoter, and the T allele, over-represented in OA, shows reduced transcriptional activity. This SNP is associated with susceptibility to knee and hip OA in the Japanese and Han Chinese populations. However, this association was not evident in subsequent epidemiologic studies in Spanish and Greek populations. In a recent meta-analysis on the association between SNP rs143383 and OA, it was suggested that the association between the rs-143383 SNP and OA had global relevance. These reported ethnic variations led us to examine the association between GDF5 and OA of the knee in the Korean population. Our objective was to assess the relationship of the rs143383 SNP with susceptibility to knee OA development in a sample of the Korean population.

**MATERIALS AND METHODS**

Tissue samples

Degenerative articular cartilage, meniscus, and ligament tissue specimens were obtained from 276 patients with OA who had undergone total knee arthroplasty at St. Mary’s Hospital, The Catholic University of Korea in Seoul between 2004 and 2005. The patients with OA included 50 men (18.1%) and 226 women (81.9%), with a mean age of 63-years at the initial diagnosis. All patients with OA were confirmed by radiology and pathology to have degenerative joint disease. Because only patients who had undergone total knee arthroplasty were included in this study, our specimens were derived from patients with Kellgren and Lawrence grade (KL grade) four, or joint space narrowing (JSN) grade four or higher OA. As in other studies, we excluded patients with rheumatoid arthritis, polyarthritis-associated autoimmune disease, post-traumatic OA, and infection-induced OA. Patients who had clinical and radiographic findings suggestive of skeletal dysplasia were also excluded. The exclusion criteria contained other malignant diseases such as bone tumors, secondary metastases, alcohol or drug abuse, hepatic failure, and renal failure. The healthy control group consisted of 135 females and 163 males with a mean age of 44-years. We excluded individuals with symptoms of joint pain, those who were limp and had limitations in joint movement, and those with radiographic signs of JSN and formation of osteophytes. Both the controls and patients with OA belonged to the same ethnicity and geographical area. This study was approved by the Institutional Review Board (IRB) of the Catholic University of Korea, College of Medicine (IRB approval number CUMC10U029).

DNA extraction

Degenerative joint tissues were ground to a very fine power in liquid nitrogen, using a mortar and pestle, suspended in lysis buffer, and treated with proteinase K. DNA was extracted using phenol-chloroform-isooamyl alcohol and ethanol precipitation, as described previously. For the control population, a leukocyte cell pellet from each blood sample was obtained from the buffy coat by centrifuging 2 mL of whole blood. The cell pellet was used for DNA extraction. The Qiagen DNA Blood Mini kit (Qiagen, Valencia, CA, USA) was used to obtain genomic DNA, according to the manufacturer’s instructions. DNA purity and concentration were determined with a Nanodrop® ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA).

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

A PCR-RFLP assay was used to identify the GDF5 5′ UTR SNP rs143383 genotypes with 5′-AGCACACGGCAGCAT-TACG-3′ and 5′-CCAGTCCCCATAGTGAAATG-3′ primers. The 197-bp target DNA fragment contained the CGC/CCC site of the rs143383 SNP. The 10 µL PCR mixture contained 1 µL of template DNA, 0.5 µM of each primer, 0.2 µM of each deoxynucleotide triphosphate, 1.5 mM MgCl₂, 0.4 units of Taq polymerase, and 1 µL of 10× buffer. The reaction mixture was denatured for 12 minutes at 94°C and incubated for 35 cycles (denaturing for 40 seconds at 94°C, annealing for 40 seconds at 57°C, and extension for 40 seconds at 72°C). The final extension was continued for 5 minutes at 72°C. The SNP alters a BsiEI restriction enzyme recognition site and was genotyped using a PCR-restriction enzyme analysis. The 197-bp fragment was then digested with 5 units BsiEI (New England Biolabs Inc., Ipswich, MA, USA) for 4 hours at 60°C. The digested product was separated on a 3% agarose gel with ethidium bromide and photographed with an Ultra Violet Product Image Storage system. The T/T genotype produced a single 197-bp band due to absence of the BsiEI restriction site; the C/C genotype produced...
two bands (106 bp and 91 bp), and the C/T genotype produced three bands (197 bp, 106 bp, and 91 bp) (Fig. 1). The results were evaluated by one of the authors blinded to the status of the study cohort. More than 10% of the samples were selected randomly for repeated assay, and the results were 100% in agreement.

Statistical analysis

The chi-square test for association was used to test differences of in the genotype or allele frequencies between patients with OA and healthy controls. Genotype-specific risks were estimated as odds ratios and 95% confidence intervals using multiple logistic regression.

RESULTS

The genotype frequencies of the +104T/C polymorphism in Korean patients with knee OA and the controls are summarized in Table 1. The frequencies of the TT, CT, and CC genotypes in normal healthy individuals were 53.4% (159/298), 37.9% (113/298), and 8.7% (26/298), respectively. The frequencies of the T and C alleles were 72.3% and 27.7% in healthy individuals, respectively. The genotype frequencies of both groups were consistent with those previously reported in the Japanese and Han Chinese populations. For patients with OA, the TT, CT, and CC genotypes had a prevalence of 54.3% (150/276), 41.7% (115/276), and 4.0% (11/276), respectively, and the T and C allele frequencies were 75.2% and 24.8%, respectively. No significant difference in genotype or allele frequencies of the +104-T/C SNP of the GDF5 gene was observed between the cases and controls (p = 0.0631 and p = 0.2705, respectively).

The associations between the +104T/C polymorphism genotype and OA stratified by age and gender are shown in Table 2. Because the most common age for OA to occur in Koreans is approximately 50 years old, we classified the patients into two age groups: “young” patients (<50 years old) & “old” patients (>50 years old). Unexpectedly, no significant differences were observed when patients were stratified by age and gender (p = 0.4862 and p = 0.2286, respectively).

DISCUSSION

Because OA has an established genetic background, identifying susceptibility genes is the most promising approach to understand the disease, as it helps to elucidate the primary biological events causing OA. A number of OA susceptibility genetic loci, including ASPN, CALM1, COL2A1, COMP, and FRZB, have been reported, and some play a role causing OA in more than one study. However, identifying alleles associated with a high OA risk is complicated, due to the complexity of the tissues involved in joints and multiple genetic factors.

Table 1. Distribution of the rs143383 single nucleotide polymorphism genotype and frequency in patients with osteoarthritis and controls

<table>
<thead>
<tr>
<th>rs143383</th>
<th>Cases (n = 276)</th>
<th>Controls (n = 298)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusteda OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>CT</td>
<td>150</td>
<td>54.3</td>
<td>159</td>
<td>53.4</td>
</tr>
<tr>
<td>CC</td>
<td>11</td>
<td>4.0</td>
<td>26</td>
<td>8.7</td>
</tr>
<tr>
<td>T : C allele frequencyb</td>
<td>415 : 137</td>
<td>431 : 165</td>
<td>1.164 (0.891-1.521)</td>
<td>1.034 (0.708-1.511)</td>
</tr>
</tbody>
</table>

*aAdjusted for age (in year) and gender; bTwo-sided χ²-test; for allele frequencies, p = 0.2705; for genotype distribution, p = 0.0631; cCalculated in the logistic regression model using the number of T alleles in the genotype as a continuous variable.

OR, odds ratio; CI, confidence interval.
such as incomplete penetrance, variable expression, and a high degree of etiological heterogeneity between populations that may interact to influence phenotypic expression. For example, Kizawa et al.\textsuperscript{21} reported a positive association between knee OA and the D14 allele of aspartic acid (D) repeat polymorphism (ASPN) in the asporin gene. In our previous study, we found a significant association between the D13 allele and Korean patients with OA.\textsuperscript{22} Although there are ethnic differences in allelic frequency of the asporin gene, the association between ASPN and knee OA appears to be global. Nevertheless, large-scale case-control association scans and genome-wide association scans are beginning to improve our understanding of the basic causes of OA. These scans will have a direct impact on the development of new treatments for this complex and debilitating disease.

The role of GDF5 in the development and maintenance of bone and cartilage has been recognized for some time. GDF5 is known to have a critical role in the development and maintenance of bone and cartilage.\textsuperscript{23-25} Furthermore, GDF5 regulates early cartilage differentiation by promoting chondroprogenitor cell aggregation and promotes osteogenic differentiation and angiogenic activity of rat fat-derived stromal cells \textit{in vitro}, suggesting that several distinct regulatory mechanisms may exist to control osteogenic differentiation.\textsuperscript{26,27} A function for GDF5 in the etiology of OA appears highly plausible. Because the SNP in the 5′ UTR of GDF5 (+104T/C; rs143383) influences transcriptional activity in the GDF5 gene core promoter, and the T allele shows reduced transcriptional activity,\textsuperscript{5} this SNP may be the biological basis for the change in function. Recently, an association between hip and knee OA and the rs143383 SNP of GDF5 has been reported in Japanese and Chinese cohorts.\textsuperscript{6} However, no significant differences in allelic and genotypic frequencies of the rs143383 SNP of GDF5 were found in Greek Caucasians.\textsuperscript{13} As different populations have unique environmental and genetic backgrounds, we examined the association of the rs143383 SNP of GDF5 with knee OA in a Korean population sample.

In the present study, we found no significant difference in the frequency of the rs143383 genotype between healthy controls and patients with OA (p = 0.5681). Our results were inconsistent with the findings of a Japanese group, which reported an association between rs143383 and OA in the Japanese (p = 0.0021) and Han Chinese (p = 0.00028) populations.\textsuperscript{7} Although rs143383 genotype frequencies of GDF5 in the Korean population were consistent with those of the Japanese and Chinese populations, sample size alone was not the explanation for this discrepancy. Instead, a difference in the criteria used for patient enrollment likely accounted for this discrepancy. Although the Japanese and Chinese patients were of KL grade two or above, this study contained more terminal OA cases (KL grade 4 or JSN grades 4 or 5). Therefore, the degree of OA severity was different. Further studies in a larger population with similar inclusion criteria and disease classification are crucial to elucidate the role of GDF5 as a susceptibility gene in knee OA. In addition, it has been reported that GDF5 expression is influenced by a second 5′ UTR rs143384 SNP.\textsuperscript{28} Another polymorphism, located in the 3′ UTR of GDF5, influences allelic expression of the gene, independent of rs143383.\textsuperscript{28} It is necessary to identify other possible variants within the GDF5 locus that have large, singular effects on GDF5 expression. Furthermore, differences between ethnic groups have been found in SNPs of other genes relating to OA susceptibility, such as asporin\textsuperscript{21,22,29} and LRCH1.\textsuperscript{30} These results imply the existence of other polymorphic loci, different sampling criteria, or cultural and environmental factors that can influence OA susceptibility between different ethnic groups. Because the rs143383 SNP of GDF5 is not a risk factor for OA etiology in Koreans and Greek Caucasians,\textsuperscript{13} it is

### Table 2. Subgroup analysis of the rs143383 single nucleotide polymorphism genotype frequency in patients with osteoarthritis and controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>rs143383 genotype</th>
<th></th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>No. of controls</td>
<td>TT vs CT</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>TT</td>
<td>CT</td>
<td>CC</td>
</tr>
<tr>
<td>≤ 50</td>
<td>19</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>131</td>
<td>95</td>
<td>10</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>Female</td>
<td>127</td>
<td>89</td>
<td>10</td>
</tr>
</tbody>
</table>

*Adjusted for the other covariate (age [in year] as a continuous variable) presented in this table in a logistic regression model for each stratum. OR, odds ratio; CI, confidence interval.
likely that the rs143383 GDF5 core promoter polymorphism might be specific to the Chinese and Japanese.

In summary, our study did not demonstrate an association between the +104T/C GDF5 polymorphism and knee OA in the Korean population. Furthermore, it did not validate previous positive findings of the Japanese group but rather emphasized the necessity of independent large-scale studies to clarify the effect of this polymorphism in OA pathogenesis.

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