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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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Background : Osteoarthritis (OA) is a common disease characterized by degenerating joint cartilage in the knee, hip, and hand. A functional single nucleotide polymorphism (SNP) +104-T/C; rs143383 in the 5′ untranslated region of the growth differentiation factor 5 (*GDF5*) gene was recently associated with susceptibility to OA in the Japanese and Chinese populations. **Methods :** To investigate whether this association is present in the Korean population, the frequency of the polymorphism was investigated in 276 patients with knee OA and 298 healthy subjects as controls. Polymorphism analysis was performed by amplifying the core promoter region of the *GDF5* gene and digesting it with the *BsiEI* restriction enzyme. **Results :** The frequency of the TT, CT, and CC genotypes was 54.3% (150/276), 41.7% (115/276), and 4.0% (11/276), respectively, in patients with OA, and 53.4% (159/298), 37.9% (113/298), and 8.7% (26/298), respectively, in healthy controls. No significant differences in genotypic or allelic frequencies of the +104T/C SNP of the *GDF5* gene were observed between patients with OA and controls. Also, no significant differences in allelic and genotypic frequencies were found when the individuals were stratified by age and gender. **Conclusions :** The results suggest that the +104T/C; rs143383 *GDF5* core promoter polymorphism is not a risk factor for OA in the Korean population.

Key Words : Growth differentiation factor 5; Osteoarthritis; Polymorphism; Susceptibility; Korean population

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.[7,8] *GDF5* mutations have been implicated in several skeletal development disorders, such as various forms of chondrodysplasia, synphalangism, and type C brachydactyly.[9] In addition, transgenic mouse studies suggest that GDF5 promotes differentiation of chondrocytes, causing hypertrophy, and enhances commitment of mesenchymal cells to the chondrocyte lineage.[10] GDF5 is present in both normal and osteoarthritic articular cartilage, and responsiveness to GDF5 is preserved in

osteoarthritic chondrocytes.[11] 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.[8] This SNP is associated with susceptibility to knee and hip OA in the Japanese and Han Chinese populations.[6] However, this association was not evident in subsequent epidemiologic studies in Spanish and Greek populations.[12,13] In a recent meta-analysis on the association between SNP rs143383 and OA, it was suggested that the association between the rs143383 SNP and OA had global relevance.[14,15]

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.[16] 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-isoamyl alcohol and ethanol precipitation, as described previously.[17] 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′-AGCACACAGGCAGCATTACG-3′ and 5′-CCAGTCCCATAGTGGAAATG-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 $MgCl_2$, 0.4 units of Taq polymerase, and 1 μL of 10× buffer. The reaction mixture was denatured for 12 minutes at 94℃ and incubated for 35 cycles (denaturing for 40 seconds at 94℃, annealing for 40 seconds at 57℃, and extension for 40 seconds at 72℃). The final extension was continued for 5 minutes at 72℃. 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℃. 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.[8] 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).

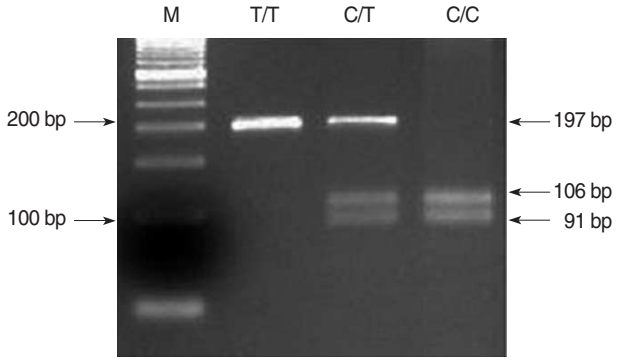

Fig. 1. Genotype analysis of the rs143383 single nucleotide polymorphism of growth differentiation factor 5. Restriction fragment length polymorphism patterns of the heterozygote C/T and homozygotes T/T and C/C.

DISCUSSION

Because OA has an established genetic background,[4,5] 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.[18-20] 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

rs143383	Cases (n = 276)		Controls (n = 298)		Crude OR (95% CI)	Adjusted[a] OR
	n	%	n	%		
TT	150	54.3	159	53.4	1.00	1.00
CT	115	41.7	113	37.9	1.079 (0.766-1.519)	1.213 (0.746-1.974)
CC	11	4.0	26	8.7	0.449 (0.214-0.940)	0.574 (0.205-1.612)
T : C allele frequency[b]	415 : 137		431 : 165			
Trend test[c]					1.164 (0.891-1.521)	1.034 (0.708-1.511)

[a]Adjusted for age (in year) and gender; [b]Two-sided χ^2-test; for allele frequencies, p = 0.2705; for genotype distribution, p = 0.0631; [c]Calculated in the logistic regression model using the number of T alleles in the genotype as a continuous variable.
OR, odds ratio; CI, confidence interval.

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

Variable	rs143383 genotype						Adjusted[a] OR (95% CI)	
	No. of cases			No. of controls				
	TT	CT	CC	TT	CT	CC	TT vs CT	TT vs CC
Age (yr)								
≤ 50	19	20	1	132	88	23	1.567 (0.790-3.108)	0.304 (0.039-2.388)
> 50	131	95	10	27	25	3	0.962 (0.492-1.883)	0.591 (0.134-2.602)
Gender								
Male	23	26	1	89	58	16	1.497 (0.737-3.040)	0.290 (0.035-2.384)
Female	127	89	10	70	55	10	1.244 (0.615-2.498)	0.732 (0.175-3.066)

[a]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.

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.*[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.[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 expressed in regions of future joints during early development, and *GDF5* mutations have been associated with abnormal joint development.[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 *in vitro*, suggesting that several distinct regulatory mechanisms may exist to control osteogenic differentiation.[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,[6] 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.[6] However, no significant differences in allelic and genotypic frequencies of the rs143383 SNP of *GDF5* were found in Greek Caucasians.[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.[8] Although rs143383 genotype frequencies of *GDF5* in the Korean population were inconsistent 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.[28] Another polymorphism, located in the 3′ UTR of *GDF5*, influences allelic expression of the gene, independent of rs143383.[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*[21,22,29] and *LRCH1*.[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,[13] it is

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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