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Original Research

Is *GDF5* gene promoter polymorphism +104T/C associated with osteoarthritis in the Eastern of Turkey population?

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Abstract: Osteoarthritis (OA) is the most common form of arthritis. Genetic factors have been shown to play important roles in the etiology of OA. The gene growth differentiation factor 5 (*GDF5*) has been implicated in skeletal development and joint morphogenesis in human and mice. A functional single nucleotide polymorphism (SNP) +104T/C in the 5'-UTR of *GDF5* (rs143383) was reported to be associated with osteoarthritis susceptibility in Han Chinese and Japanese populations. Our objective was to assess whether this SNP was also associated with OA in the Eastern Turkey population.A total of 172 cases including 95 patients with idiopathic OA and 77 control cases were recruited into the study. DNA samples were extracted from peripheral blood lymphocytes of all cases by using salting out method. The +104T/C polymorphism was genotyped by PCR-RFLP method. In terms of genotype comparison there wasn't any correlation between patient and control groups. Frequency of C allele was found to be higher in-patient group than control group and statistical analysis showed a poor correlation in allele frequencies of the +104T/C SNP of *GDF5* gene between cases and controls ($p<0.05$). Significant correlation between *GDF5* and OA has been reported in Asian population, especially T alleles were found in higher frequencies and related to OA. Our study did not confirm this association and also in term of T allele. Interestingly, we found higher frequency of C allele in patient group than control group and our results are compatible with the study carried out in Greek population.

Key words: Osteoarthritis; *GDF5*; SNP; Polymorphism; PCR-RFLP.

Introduction

Osteoarthritis (OA) can be defined a degenerative disorder that hold the entire joint structures, including the cartilage, joint lining, ligaments, subchondral bone and synovium (1). Idiopathic OA is also called primary OA is occurring in previously intact joints without any identifiable cause, such as injury of the joint or developmental abnormalities (2). Disturbance of balance between degradation and synthesis of articular joint tissues especially articular cartilage considered to be involved in disease process (3,4). In the progress of OA, increased water content after the loss of matrix strength and disrupted extracellular matrix (proteoglycan, aggrecan, collagen), fiber fibrillation and surface splits reported (4). Matrix metalloproteinases play crucial roles in the degradation of extracellular matrix in OA (5,6).

Genetic predisposition to OA and role of the polymorphisms in the pathogenesis of OA have demonstrated by previous studies (2,6-9). According to the literature, a functional polymorphism located atthe 5' UTR regionof *GDF5* gene, was associated with sensitivity of knee and hip osteoarthritis in Han Chinese and Japanese populations (10). It has been proposed that this polymorphism negative affect the transcriptional activity of the *GDF5* gene (11). It has been shown that *GDF5* is important in regulation of matrix metalloproteinases and also has shown matrix metalloproteinases are important catabolic enzymes that take role in degradation of the collagen (6,12).

In this study, we aimed to determine the genotype and allele frequencies of SNP located in the 5'-UTR of *GDF5* gene in healthy subjects and OA patients and to identify whether is there a relationship between OA in the Eastern of Turkey population and this SNP.

Materials and Methods

Unrelated 95 osteoarthritis patients that admitted to Physical Medicine and Rehabilitation (PM&R)/Rheumatology clinic of Yuzuncu Yil University Faculty of Medicine and gender and age matched 77 healthy volunteers were enrolled into the study. All individuals informed about the study tests and inspections related to systemic diseases and osteoarthritis were carried out. DAS-28 inquiry form was filled to patients as related to the disease activity. Peripheral blood samples of all participants were taken in to the anticoagulant tube and stored at -20 °C.

Patients and control groups

This study planned as a case-control study including a patient group consisting of 95 OA patients and a control group consisting of healthy 77 volunteers. Each group were separated according to age and gender and body-mass index was calculated by measuring height and weight. Patients with rheumatoid arthritis, with chondrodysplasia, infection-induced OA and postrheu-

matic OA were excluded from the study. Studied population was in Hardy-Weinberg equilibrium and ethnic homogeneity.

Genotyping

Genomic DNA samples were extracted from peripheral blood leukocytes by using salting out method. PCR amplifications were performed by primers designed according to the *GDF5* gene sequence given in previously published report (13). Primer sequences were as follows; Forward primer:5'-AGCACACAGGCAGCATTACG -3' and Reverse primer:5'-GCCTCTCCTTGGCCTCTG-3'. PCR conditions were denaturation at 94 °C for 5 minutes followed by 40 cycles of following conditions: denaturation at 94 °C for 30 sec, annealing at 60 °C for 30 sec and extension at 72 °C for 30 sec and one cycle of at 72 °C for 10 minutes. Final PCR amplification product was 532 bp in length. Amplicons were digested with Fast Digest BsiEI enzyme (Thermo Scientific) for 5 minutes in 37 °C and heat inactivation carried out in 80 °C for 15 minutes. Digested products were electrophoresed in 2% agarose (A9539 SIGMA) after staining with ethidium bromide. Images visualized with Quantum ST4 (VilberLourmat). The amplified PCR product was 532 bp and after digestion with the enzyme, homozygote C allele was shown two bands (106 bp and 426 bp), in the case of heterozygote (C and T allele) there were three bands (532+426+126 bp) in the case of homozygote T allele there was just a 532 bp band.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Chisquare test was used to test the significance of distribution of OA patients and healthy controls. Descriptive statistics were expressed as count and percent. $p< 0.05$ was considered statistically significant.

Results

Ninety fine patients with idiopathic OA (80% female and 20 % male) and Seventy-seven healthy control subjects (60% female and 40 % male) were enrolled in this study. The mean age of the study group was 62,5±8,4 years (min: 43 years; max: 76 years) in match with the mean age of the controls 61,9±5,3 years (min: 44 years; max: 75 years) ($p > 0.05$).

Distribution of genotype frequencies and results of statistical analysis of *GDF5* polymorphism of OA patients and healthy controls are summarized in Table 1. Statistical analysis of genotypes have shown that there weren't any statistical differences between patients and control groups.

Assessment of allelic frequencies showed C allele is more than T allele in patient group than control group. Statistical analysis showed a poor correlation of allele frequency between patients and control groups. Results summarized in Table 2.

Discussion

GDF5 is an extracellular signaling molecule belongs to the TGF-beta super family, and it takes part in development of especially cartilage and other tissues of synovial joint (14,15). It is also known as cartilage-derived morphogenetic protein-1 (CDMP1) (16). Importance of *GDF5* in OA is firstly reported in Japanese and Chinese populations (10). Significant correlations of the SNP (rs143383), T to C transition located in the 5' untranslated region of the *GDF5* gene between patient and control groups have been shown in the studyby Miyamoto et al (2007) (10). Furthermore, low expression of the *GDF5* protein related to the T-allele of SNP rs143383 has shown (11). Also, expression of *GDF5* have been shown to inhibit catabolic MMP13, it is important in destruction of cartilage, and stimulate anabolic SOX9 and ACAN expression in human articular cartilage (8).

Egli et al (2009) proved significantly decreased expression of T allele compared to C allele in all tissues (cartilage, fat pad, ligament and meniscus) of OA patients obtained during the joint-replacement surgery. Southam et al showed a 27% reduction of *GDF5* expression in individuals carrying T allele in the cartilage of OA patients who had undergone joint-replacement surgery and also demonstrated a correlation when compared the allele frequencies between in a broad OA patients and control groups (17). Studies carried out by Miyamoto et al (2007) (10), Tawonsawatruk et al (2011) (18), and Mishra et al (2013) (19), suggested a positive correlation between T allele and OA patients while Tsezou et al (2008)(13), Shin et al (2012)(20), and Cao et al (2010) (21) have not found any correlation between T allele and OA in their studies. Like last these three studies, we have not able to show a positive correlation between T allele and OA patients and control group. It should be noted our results are closely similar to result obtained by Tsezou et al (2008) in Greek population (13).

Pan et al (2014) showed T allele of *GDF5* is associated with a higher risk for knee OA development and

Table 1. Genotype frequencies and χ^2 values.

Genotypes	Patients N=95	Controls N=77	Genotype frequencies of patients	Genotype frequencies of controls	χ^2
TT	32	30	0,34	0,39	0,32
TC	39	39	0,41	0,51	0,85
CC	24	8	0,25	0,10	4,45

Table 2. Allele frequencies and statistical analysis of patients and control groups.

Alleles	Patients N=95	Controls N=77	OR (95% CI)	χ^2	P value
T allele	103	99	0,658 (0,425-1,017)	3,58	0,038
C allele	87	55			

C allele has a protective role for knee OA susceptibility by the meta-analysis performed on 23,995 individuals (22). Interestingly, subgroup analysis performed in this metaanalysis on ethnicity showed strong significant association in Asian population and weak association in Caucasian population.

In our study, we could not observe a statistically significant correlation between rs143383 polymorphism and OA susceptibilityin our study population. This result is compatible with the studies carried out in Greek, Korean and Thai populations. As discussed above in the perspective of ethnicity, this locus is the only locus successfully validated across diverse Asian and European populations. Studying the other candidate genes in larger populations will contribute more benefits to understanding the OA pathophysiology.

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Conflict of interest statement
The authors declare no conflict of interest.

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