INTRODUCTION

Osteoarthritis (OA) is a widespread, polygenic multifactorial disease characterised by joint cartilage degeneration and synovial inflammation.\(^1\) Knee OA (gonarthrosis), one of the most frequently encountered types of OA, is the chronic degeneration of the joint cartilage, caused by the disruption of the soft tissues around the joint.\(^2\) The common risk factors for OA are obesity, diabetes, previous joint injury or disease, and genetic and anatomic abnormalities. Furthermore, there is also said to be an association with several genetic polymorphisms.\(^3\)

Studies have shown that the growth differentiation factor 5 (GDF5) gene takes a significant role in musculoskeletal system disease, endochondral ossification, synovial joint formation, tendon repair and bone production.\(^4-7\) Mutations in this gene have been shown to cause skeletal disorders or the development of abnormal joints in both mice and humans.\(^8\) The transcriptional activity in the GDF5 gene core promoter is affected by the +104T/C polymorphism (rs143383) in the 5′-untranslated region (UTR) of GDF5 gene, and it has been shown that transcriptional activity decreases in individuals carrying T alleles.\(^9\)

Furthermore, GDF5 mutation has been found to play a role in patients with some type C brachydactyly and angel-shaped phalango-epiphyseal dysplasia.\(^10,11\) In both disorders, joint OA is a common symptom. The T allele of the single nucleotide polymorphism (SNP) is associated with an increased risk of OA in European and Asian populations.\(^12\) These findings suggest that GDF5 has a significant role in the etiology and pathogenesis of OA.\(^13,14\)

There has been no previous study on a Turkish population that has investigated whether or not polymorphism

ORIGINAL ARTICLE

Polymorphisms in the Growth Differentiation Factor 5 (GDF 5) Gene in Knee Osteoarthritis

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ABSTRACT

Objective: To identify the frequency of the rs143383 SNP in the GDF5 gene, which is located in the 5′-untranslated region of Turkish population with knee osteoarthritis (OA).

Study Design: A case-control study.

Place and Duration of Study: Orthopedics and Traumatology Department, Bozok University Medical Faculty, Yozgat, Turkey, from 2012 to 2014.

Methodology: Patients diagnosed with OA (n=94) and patients who did not have joint complaints (n=279) were enrolled in this study. Patients diagnosed with osteoarthritis according to the 1986 American College of Rheumatology osteoarthritis criteria and Kellgren and Lawrence scores were investigated, based on age, gender, and X-ray findings. Blood samples were taken for the identification of GDF5 (rs143383) SNPs by PCR/RFLP, according to a standard protocol.

Results: This study included 373 patients. The OA group (25.2%; n=94) was characterized by specific genotypes: TT (39.4%; n=37); heterozygotes (TC; 45.7%; n=43); and homozygotes (CC; 14.9%; n=14). The control group (74.8%; n=279) was comprised of TT (26.5%; n=74), TC (54.8%; n=153), and CC (18.6%; n=52) genotypes. An analysis of rs143383 SNP of the GDF5 gene polymorphism revealed that the rs143383 TT genotype had a higher risk for OA (crude OR=1.798, 95% CI=1.010-2.941, p=0.021).

Conclusion: This study demonstrated that there is a correlation of +104T/C polymorphism in the 5′-UTR of GDF5 with knee OA in a Turkish population.


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(rs143383) of the GDF5 gene is associated with knee OA in Turkey. Hence, the aim of this study was to analyse the relationship of the +104T / C polymorphism in the GDF5 gene with gonarthrosis.

METHODOLOGY

The study was performed from 2012-2014 at Department of Orthopedics and Medical Biology the Bozok University Medical Faculty, Yozgat, Turkey. The participants were Turkish nationals living in Turkey for at least 3 generations. A total of 373 persons were included in this case-control association research. G Power 3.1.92 software program was used to obtain the G power value for the study groups. The number of participants required to achieve an actual power value greater than 0.804 was 279 in the control group and 94 in the patient group (actual power value = 0.9868).

Clinical and radiological diagnostic criteria were based on the American College of Rheumatology criteria (1986) and the Kellgren and Lawrence scores, respectively.15,16 The patient (study) group was selected from patients with Grade 3 and/or 4 knee plain radiographs and a diagnosis of OA. The control group was selected from individuals for whom knee plain radiographs were performed because of any other complaint. The exclusion criteria were age less than 50 years; history of bone fracture in the knee; diagnosis or treatment history of inflammatory arthritis (e.g., rheumatoid arthritis); or any hematologic disorder. Informed consent form was obtained from each subject, and the study was approved by the Ethical Committee of the Bozok University Faculty of Medicine [18.03.2012/11-10].

Peripheral venous blood samples of 3 mL were drawn from each individual by a standard vein puncture. Genomic DNA was extracted from peripheral blood leukocytes of 94 OA patients and 279 controls, using the commercially available Qiagen DNA Blood Mini kit. For polymorphisms and genotyping rs143383 single nucleotide polymorphism was screened in the GDF5 gene using the samples. Polymorphisms were genotyped using the PCR-RFLP method with BsiEI restriction enzyme. PCR primers were designed by the Primer-3 web-based tool: A 197 bp DNA region encompassing rs143383 was amplified by PCR using 5'-AGCACACAGGCAGCATTACG- 3' and 5'-CCAGTCCCATAGTGGAAATG-3'. PCR amplicons were digested with BsiEI restriction enzyme. Restriction patterns were visualized on 2% agar gels stained with ethidium bromide. The expected fragment length was 106 and 91 bp in CC, 197, 106, and 91 bp in TC, and 197 bp in TT genotypes.

The unpaired t-test was used for continuous data and Chi-square was used for categorical data. Multinominal logistic regression analyses were applied for determining the effects of genotypes and allele frequency on the severity of knee osteoarthritis. The odds ratios and 95% confidence intervals, which were crude and also after adjustment for age and gender, were calculated. Data analysis was performed by using IBM SPSS Statistics version 17.0 software (IBM Corporation, Armonk, NY). A p-value less than 0.05 was considered as statistically significant.

RESULTS

Of the 373 subjects, 94 (25.2%) were cases and 279 (74.8%) were placed in the control group. Table I shows the demographics of the study of case and control groups. There was no significant difference between the two groups according to their average ages (Table I). The distribution of genotype frequencies of the study and control groups is shown in Table II. Association analysis revealed that GDF5+104T/C was associated with OA. In the OA group 25.2% (n=94), the frequencies of the TT, TC, and CC genotypes at the -1486T/C SNP were 39.4% (n=37), 45.7% (n=43), and 14.9% (n=14), respectively. In the control group 74.8% (n=279), the frequencies of the TT, TC, and CC genotypes at the +104T/C SNP were 18.6% (n=52), 54.8% (n=153), and 26.5% (n=74), respectively (Table II). rs143383 TT genotype appeared to have a higher risk for OA (crude OR=1.798, 95% CI=1.010-2.941, p=0.021, Table II).

Thus, the analysis demonstrated that the +104T/C polymorphism in the GDF5 gene was associated with the risk of knee OA in a Turkish population (Table II).

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>Control (n=279) (%)</th>
<th>Case (n=94) (%)</th>
<th>Crude OR (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>74 (26.5%)</td>
<td>37 (39.4%)</td>
<td>1.798 (1.010-2.941)</td>
<td>0.021</td>
</tr>
<tr>
<td>TC</td>
<td>153 (54.8%)</td>
<td>43 (45.7%)</td>
<td>0.694 (0.434-1.110)</td>
<td>0.127</td>
</tr>
<tr>
<td>CC</td>
<td>52 (18.6%)</td>
<td>14 (14.9%)</td>
<td>0.764 (0.402-1.453)</td>
<td>0.404</td>
</tr>
<tr>
<td>TT+TC</td>
<td>205 (73.4%)</td>
<td>57 (60.6%)</td>
<td>0.556 (0.340-0.909)</td>
<td>0.019</td>
</tr>
<tr>
<td>TT+CC</td>
<td>227 (81.3%)</td>
<td>80 (85.1%)</td>
<td>0.764 (0.402-1.453)</td>
<td>0.404</td>
</tr>
</tbody>
</table>

OR = Odds ratio, CI = Confidence interval.

Table II: The association between each genotype.
**DISCUSSION**

GDF5 is a gene which plays an important role promoting the development, care and repair of synovial joint tissues in the cartilage and joints in particular. The GDF5 gene has a role in musculoskeletal systems and has a role, especially in the endochondral ossification process in cartilage tissue. Since GDF5 was chosen as an OA candidate gene phalangeopipheal dysplasia, patients develop severe and early-onset hip OA, as observed in a number of populations. The expression of the GDF5 protein is modulated by the GDF5 gene, and rare deleterious mutations in the GDF5 gene cause various disorders of skeletal development, such as brachydactyly and chondrodysplasias. These studies show that GDF5 gene takes an important role in joint homeostasis and repair.

Studies conducted on individuals with OA have shown that polymorphism in rs143383 in the promoter region of GDF5 reduces transcriptional capability, suggesting that this variant affects the GDF5 transcription. This relationship has been shown for knee OA in both Japanese and Han Chinese populations; and two recently published meta-analyses have confirmed it in a European population. In a previous meta-analysis of European and Asian populations by Zhang et al., a significant relationship was observed between GDF5 rs143383 polymorphism, and especially knee and hand OA, with the relationship determined for knee OA in 7965 European and 12747 Asian patients, for hip OA in 6363 European and 9727 Asian patients, and for hand OA in 4335 European and 5991 Asian patients. In another meta-analysis by Pan et al., which included 23995 individuals, an association was found between knee OA risk and GDF5 rs143383 polymorphism. While several studies have demonstrated a relationship between OA and GDF5, other groups have not confirmed these results. The results of the current study revealed that SNP rs143383 of GDF5 is a compelling risk factor for knee OA and that GDF5 has an etiological effect on the development of OA.

Genetic studies related to OA have increased in recent years and these studies are important in respect of understanding the etiology of the disease and finding early resolutions. The susceptibility to complex diseases varies according to race and ethnicity, as well as environmental factors, thus affecting the genetic contributions to susceptibility. There is a correlation of +104T/C polymorphism in the 5'-UTR of GDF5 gene polymorphism with knee OA in a Turkish population. To increase the understanding of OA's genetic component, further studies are required on other genes and polygenic inheritance, and it is important to identify new genetic risk factors. Larger samples and multi-site projects are required, all of which we lacked due to limited funding.

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