Polymorphisms in *ADAMTS4* and *ADAMTS5* are not linked to susceptibility to knee osteoarthritis in the Turkish population

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ABSTRACT. Considering the functions of aggrecanase-1 (ADAMTS4) and -2 (ADAMTS5), which are thought to be the two major enzymes responsible for the destruction of aggrecans in arthritic diseases, we investigated whether important polymorphisms in the *ADAMTS4* and *ADAMTS5* genes affect osteoarthritis (OA) susceptibility. Our study took place in Mugla, Turkey. Ninety-five cases were recruited following OA diagnosis (72 women and 23 men), and 80 individuals without any symptoms or radiographic signs of OA (56 women and 24 men) were chosen as healthy controls. After obtaining DNA from

patients and control subjects, *ADAMTS4* and *ADAMTS5* genotypes were determined using the ABI Prism StepOnePlus Real-Time system. In addition, we categorized patients based on OA grade. There were no significant differences in the genotype distributions of the four polymorphisms between the groups ($P > 0.05$). Moreover, *ADAMTS4* and *ADAMTS5* allele frequencies did not differ between OA and control participants ($P > 0.05$). These findings suggest that the *ADAMTS4* (rs4233367 and rs11807350) and *ADAMTS5* (rs226794 and rs2830585) variants examined may not contribute to susceptibility to knee OA in the Turkish population. Other gene polymorphisms should be assessed in order to explain variations in OA susceptibility.

Key words: Knee osteoarthritis; Polymorphism; *ADAMTS4*; *ADAMTS5*

INTRODUCTION

The prevalence of osteoarthritis (OA), the most common form of arthritis, increases with age (Forestier et al., 2011). Although OA can occur in any joint, it is most frequently seen in the knee. Knee OA is the leading cause of physical disability in elderly people, and one of the most frequent reasons for total joint replacement. The prevalence of symptomatic knee OA has been reported as 13% in adults older than 55 years (Valdes and Spector, 2011). This disease affects not only joint cartilage, but all joint structures, including subchondral bone, ligaments, capsule, synovial membrane, and periarticular muscles. Knee OA is in fact an organ (synovial joint) disorder. OA can be defined by joint changes, and efforts to correct abnormal biomechanics can be initiated as a result of mechanical damage to joints (Burnett et al., 2006).

Many members of the a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family of secreted zinc metalloproteinases are known to bind and degrade extracellular matrix components. The role of ADAMTS enzymes in OA has been very well defined (Murphy and Lee, 2005; Bondeson et al., 2008; Lin and Liu, 2010). However, although knowledge of the function and role of ADAMTS metalloproteinases has increased significantly, their genetic influence on arthritis pathogenesis is not yet fully understood (Lin and Liu, 2010; Takahashi et al., 2010).

ADAMTS4 (aggrecanase-1) and ADAMTS5 (aggrecanase-2) are the most important aggrecanases in human joint cartilage (Tortorella et al., 2001). They disrupt the structure of aggrecan, the principal cartilage proteoglycan, by breaking a connection in its core protein (Bau et al., 2002; Song et al., 2007; Prasadam et al., 2012). Although ADAMTS5 may play a major role in the development of arthritis in murine models, studies involving human tissue seem to suggest that, in fact, ADAMTS4 is the more influential molecule in human arthritis (Naito et al., 2007). In one study, genetic variation in human *ADAMTS5* did not seem to affect OA susceptibility (Rodriguez-Lopez et al., 2008); however, a separate investigation reached the opposite conclusion (Gu et al., 2013). Moreover, a non-synonymous single nucleotide polymorphism (SNP) of *ADAMTS14* (rs4747096) has been shown to be associated with knee OA in Thai women (Poonpet et al., 2013). To the best of our knowledge, no study associating *ADAMTS4* gene polymorphism with knee OA has been reported.

The aim of this case-control study was to investigate the relationship between primary knee OA and *ADAMTS4* (rs4233367 and rs11807350) and *ADAMTS5* (rs226794 and rs2830585) gene polymorphisms in the Turkish population.

MATERIAL AND METHODS

Study group

A total of 95 patients presenting at our orthopedic clinic with primary knee OA were included in this study. Anterior and posterior radiographs of the affected knee were obtained. Two examiners blinded to clinical information assessed radiographic features. Each radiograph was given a global Kellgren-Lawrence (KL) score ranging between 1 and 4, as previously described (Kellgren and Lawrence, 1957). Patients with radiographic OA and a KL score of 2 or higher were included in this study, while those with multiple-joint problems were excluded. Patients with other conditions affecting knee joints, such as inflammatory, post-traumatic, or post-septic arthritis or developmental dysplasia were excluded from the study. The control group consisted of 80 individuals over 50 years old selected from among attendees of polyclinics at the Mugla Sitki Kocman University Hospital. Control participants demonstrated no signs or symptoms of OA, other arthritic conditions, or joint diseases. The Ethics Committee of the Mugla Sitki Kocman University Faculty of Medicine approved this study, and informed consent was obtained from all participants.

Genotypic analyses of *ADAMTS4* and *ADAMTS5* polymorphisms

Blood samples (2 mL) from patients and controls were drawn into ethylenediaminetetraacetic acid-containing tubes and stored at -20°C until needed for DNA analysis. DNA was isolated using a PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA), and polymorphisms were screened with the ABI Prism StepOnePlus Real-Time system (Applied Biosystems, Foster City, CA, USA) using TaqMan probes targeting the SNPs rs4233367, rs11807350, rs226794, and rs2830585. Each polymerase chain reaction (PCR) consisted of an 11-μL mixture containing 5 μL TaqMan Genotyping Master Mix, 0.25 μL 40X TaqMan Genotyping Assay stock, 2.75 μL DNase- and RNase-free water, and 100-200 ng DNA in 3 μL. The RT-PCR protocol consisted of an initial step of 94°C for 10 min, followed by 40 cycles of 95°C for 15 s for denaturation, 60°C for 1 min for annealing. Homozygous mutant, heterozygous, and homozygous wild-type genotypes were identified according to software-based allele discrimination.

Statistical analysis

SPSS 20.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The conformity of genotype distributions to Hardy-Weinberg equilibrium was analyzed by chi-square goodness-of-fit tests. Differences in genotype and allele distributions between patients and controls were assessed by chi-square tests. Normally distributed numerical data and differences in genotype and allele distributions were assessed using one-way analysis of variance. P values <0.05 were considered statistically significant in all tests.

RESULTS

The demographic characteristics of the study population are shown in Table 1. While age and gender did not differ between groups, there was a significant difference in body

mass index (BMI; P = 0.005). The study group was categorized according to age (years) and BMI (kg/m^2) using a multiple-logistic regression model. Genotype distributions and allele frequencies of the *ADAMTS4* and *ADAMTS5* gene polymorphisms were compared between OA patients and control subjects.

Table 1. Characteristics of the study subjects.

Clinical characteristics	Controls (N = 80)	OA patients (N = 95)	P value
Age (years)	64.88 ± 9.04	65.31 ± 7.64	0.733
Female/male (%)	56/24 (70/30)	72/23 (76/24)	0.389
BMI (kg/m^2)	24.75 ± 3.36	26.18 ± 3.23	0.005
K-L score	-	0 (0.0%)	
1	-	43 (46.3%)	
2	-	32 (32.6%)	
3	-	20 (21.1%)	
4			

OA = osteoarthritis, BMI = body mass index, K-L = Kellgren-Lawrence.

The genotype distributions of *ADAMTS5* (rs226794 G/A and rs2830585 C/T) and *ADAMTS4* (rs4233367 C/T and rs11807350 C/T) polymorphisms were evaluated. The most frequently observed genotypes except for rs4233367 C/T were homozygous among both OA and control participants. Genotype distributions did not deviate from Hardy-Weinberg equilibrium, and did not significantly differ between the groups (Table 2).

Table 2. Genotype and allele distributions of *ADAMTS5* (rs226794 and rs2830585) and *ADAMTS4* (rs4233367 and rs11807350) gene polymorphisms.

	Genotype/ Allele	Patients [N (%)]	Controls [N (%)]	Chi-square	P
rs226794	GG	70 (0.71)	62 (0.762)	3.377	0.337
	GA	23 (0.24)	15 (0.20)		
	AA	2 (0.02)	3 (0.03)		
	G	163 (0.83)	139 (0.86)	4.915	0.178
	A	27 (0.14)	21 (0.13)		
rs2830585	CC	68 (0.720)	63 (0.726)	2.537	0.281
	CT	23 (0.280)	15 (0.250)		
	TT	4 (0.000)	2 (0.024)		
	C	159 (0.86)	141 (0.85)	0.057	0.810
	T	31 (0.14)	19 (0.14)		
rs4233367	CC	42 (0.442)	35 (0.449)	1.350	0.509
	CT	48 (0.505)	35 (0.449)		
	TT	5 (0.053)	10 (0.102)		
	C	132 (0.695)	105 (0.673)	0.136	0.712
	T	58 (0.305)	55 (0.327)		
rs11807350	CC	95 (1.000)	80 (1.000)	-	-
	TT	-	-		
	TT	-	-		
	C	190 (1.000)	160 (1.000)	-	-
	T				

A stratified analysis was performed to evaluate the potential association between *ADAMTS4* and *ADAMTS5* genetic variants and knee OA risk in subgroups based on gender. Neither male nor female OA patient groups significantly differed from controls in terms of genotype frequencies (P > 0.05).

Allele frequencies of the *ADAMTS4* and *ADAMTS5* gene polymorphisms were also

analyzed (Table 2). No significant differences in rs226794 G/A (P = 0.178), rs2830585 C/T (P = 0.810), rs4233367 C/T (P = 0.712), or rs11807350 C/T (not statistically tested) allele frequencies were found between patients and controls.

DISCUSSION

OA is the most common cause of functional limitation, disability, and degenerative joint disease in the elderly, and the knee is the most frequent site of primary OA (Forestier et al., 2011). Although great effort has been invested in elucidating the pathophysiology of OA, the genetic factors involved in its development remain unclear (Zhan et al., 2014). The identification of genes conferring susceptibility to OA may help to create predictive models, and thus anticipate the disease's future phenotype (Takahashi et al., 2010; Valdes and Spector, 2011).

The *ADAMTS5* gene is located on chromosome 21q21.3 and regulates the synthesis of the ADAMTS5 protein (Fosang et al., 2008; Verma and Dalal, 2011; El Khoury et al., 2013). A number of previous studies have focused on factors triggering the synthesis of this protein, and on those initiating aggrecan destruction in human joint cartilage. The association between *ADAMTS5* polymorphism and OA has not been extensively investigated (Rodriguez-Lopez et al., 2008; Gu et al., 2013). Gu et al. (2013) discovered that polymorphism of the *ADAMTS5* gene contributes to OA susceptibility in Chinese patients. Conversely, Rodriguez-Lopez et al. (2008) found no relationship between variation in this gene and OA in patients of European ancestry. The rs226794 C allele predominates in Spain, while the A allele is dominant in China (Rodriguez-Lopez et al., 2008; Gu et al., 2013). Thus, the influence of this gene on OA pathogenesis is controversial, and this potential relationship should be further investigated in different geographic locations.

This study is the first to investigate the relationship between OA and *ADAMTS5* gene polymorphism in Turkey. No differences in *ADAMTS5* rs226794 genotype and allele distributions were established between the patient and control groups. Similarly, there were no significant differences between these groups in rs2830585 genotype and allele frequencies. In addition, stratification analysis based on gender failed to reveal any significant associations between knee OA susceptibility and these genotypes.

The *ADAMTS4* gene is located on chromosome 1q31-q32 in humans. ADAMTS4 has been put forward as a biochemical marker for the recognition of early OA in many *in vivo* and *in vitro* experimental studies. However, there have been no examinations of the influence of the *ADAMTS4* gene on OA etiology in the literature (Gendron et al., 2007; Naito et al., 2007; Xue et al., 2013; Li et al., 2014). We investigated the relationship between the rs4233367 G/T and rs11807350 G/T gene polymorphisms and primary knee OA. No significant association between knee OA and rs4233367 CC, CT, or TT genotypes was established (P = 0.337, 0.281, and 0.509, respectively). It was not possible to statistically test rs11807350 genotypes in this regard, as only one allele was detected.

Some limitations to our study must be considered. Firstly, our investigation had a small sample size. Our findings need to be verified in larger groups and in different communities within Turkey. Secondly, we examined only two polymorphisms in each gene, thus possibly missing associations between other variations in these sequences and OA. Finally, our study population was not homogenous in terms of BMI, and we only considered single-joint disturbance.

In conclusion, this study found no significant relationship between knee OA and genotypes of rs226794, rs2830585, rs4233367, and rs11807350 polymorphisms. Extensive

community-based studies including other joints and different gene polymorphisms are necessary in order to verify the current findings and reveal the functional roles of these genes in OA etiology.

Conflicts of interest

The authors declare no conflict of interest.

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