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Is Sp1 binding site polymorphism within *COL1A1* gene associated with tennis elbow?

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ABSTRACT

Tennis elbow defines a condition of pain and tenderness over the lateral epicondyle of the humerus. The exact aetiology of the injury is not yet fully understood. The major constituent of tendons is type 1 collagen which is encoded by *COL1A1* gene. The aim of the study was to determine whether Sp1 binding site polymorphism (SNP rs1800012; 1546G/T) within the intronic region of *COL1A1* gene is associated with tennis elbow. One hundred and three tennis elbow patients and one hundred and three healthy subjects without any history of previous ligament or tendon injuries were recruited for this genetic association study. All participants were genotyped for the *COL1A1* Sp1 binding site polymorphism by using PCR–RFLP method. There were no observed statistical differences in the genotype ($p = 0.17$) or allele ($p = 0.11$) distributions between the groups. G allele frequency in patients and controls was 82.5% and 76.21%, and T allele frequency was 17.5% and 23.79% respectively. This study has shown that there is no association between this polymorphism and tennis elbow within the population studied.

1. Introduction

Tennis elbow, also known as "lateral epicondylitis" is characterized by discomfort and variable degrees of pain at or around the lateral epicondyle of the humerus (Kraushaar and Nirschl, 1999). Although tennis elbow (TE) is described with the terms *epicondylitis* and *tendinitis*, histopathological examinations have pointed out that TE is a noninflammatory, but a degenerative disorder, now more commonly known as tendinosis (Nirschl, 1992). Tendinopathy, as a general term for both tendinosis and tendinitis, is a widespread tendon disease, but its aetiology has not been fully clarified. Several factors have been suggested by different clinical researchers (Kannus, 1997a,b; Nirschl, 1969), but the most commonly accepted cause however is seen to be a genetic inclination to develop tendon injuries (Hakim et al., 2003; Harvie et al., 2004; Józsa and Kannus, 1997) and the multifactorial combinations of both extrinsic and intrinsic factors (Mokone et al., 2005, 2006; Riley, 2004). Gene defects affecting the formation, structure and function of the tendon fibre (collagen) may have a role in the progress, however no specific causative gene has been linked to tendinopathy (Riley, 2004).

Although tendons and ligaments are made up of different types of collagens and non-collagenous proteins, type I collagen is the main protein component of both tendons and ligaments and forms approximately 60–80% of a tendon's dry mass (Collins and Raleigh, 2009; Posthumus et al., 2009a; Silver et al., 2003). Type I collagen, a heterotrimeric molecule, is formed by two α1 chains and one α2 chain, which are the products of *COL1A1* (17q21.33) and *COL1A2* (7q22.1) genes, respectively (Myllyharju and Kivirikko, 2001; Posthumus et al., 2009a; Tilkeridis et al., 2005). The transcription of α1 chain is regulated by the promoter and the first intron of *COL1A1* gene. Sp1 transcription factor binds to the first intron which is required for enhanced transcriptional activity, and modulates gene transcription (Ghosh, 2002). In the case of a nucleotide substitution (guanine (G) to thymidine (T)) at the 1546th position of the first intron, the binding affinity of Sp1 transcription factor to this variated site increases, resulting in increased *COL1A1* gene expression and an unusual α1 chain product (Mann et al., 2001).

Sp1 binding site polymorphism has been implicated in various disorders such as lumbar disc disease (Tilkeridis et al., 2005), osteoporotic fractures (Mann et al., 2001), osteoarthritis (Lian et al., 2005), and myocardial infarction (Speer et al., 2006). The association of this polymorphism with musculoskeletal soft tissue injuries, such as cruciate ligament ruptures (Khoschnau et al., 2008), Achilles tendon ruptures

Abbreviations: *COL1A1*, collagen type 1 α1; *COL1A2*, collagen type 1 α2; *COL5A*, collagen type 5 α1; COL1α1, collagen 1 protein α1; PCR–RFLP, Polymerase Chain Reaction–Restriction Fragment Length Polymorphism; TE, tennis elbow; ACL, anterior cruciate ligament.

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and Achilles tendinopathy (Posthumus et al., 2009c) has also been investigated. There are no reports regarding the association of Sp1 binding site polymorphism with lateral epicondylitis.

As collagen type I is the major protein constituent of tendons, we hypothesize that this nucleotide variation in *COL1A1* gene, might well be related with tennis elbow.

The aim of the study was therefore to determine whether the functional Sp1 binding site polymorphism within the first intron of *COL1A1* gene is associated with an increased risk of tennis elbow.

2. Materials and methods

2.1. Participants

One hundred and three patients (seventy three women and thirty men) diagnosed with tennis elbow using clinical criteria as previously described (Sluiter et al., 2001) at the Department of Orthopaedics and Traumatology in Balikesir University Medical Faculty, were recruited for this study. The patients having symptoms of radial tunnel syndrome were excluded. One hundred and three apparently healthy participants (eighty one women and twenty two men) without any history of any tendon or ligament pathology were also enrolled as control group in the study.

To avoid any possible effects of population stratification, they were all matched for their country of birth. Participants within the patient and control groups were similarly matched for gender, age (43.67 ± 9.57 and 41.85 ± 8.34 respectively) and dominant hand (dominant hand; right, 87.38% and 85.44% respectively). Of one hundred and three patients, sixty nine had the defect in their dominant hands (67%), thirty had in non-dominant hand (29%) and four had the defect bilaterally (4%). The demographic characteristics, including age and gender of all participants in the study are summarized in Table 1.

The study was approved by Gaziosmanpasa University Ethics Committee and written informed consent was obtained from all participants. In addition, each subject completed questionnaire forms for personal details and medical history.

2.2. DNA extraction and COL1A1 genotyping

Genomic DNA for molecular analysis was extracted from blood samples of both patients and healthy people by using Gene Jet Genomic DNA Purification Kit (Fermentas, Lithuania) according to the manufacturer's protocol. DNA samples were genotyped for the Sp1 binding site polymorphism (SNP rs1800012; 1546G/T) within intron 1 of the *COL1A1* gene using PCR–RFLP (Polymerase Chain Reaction–Restriction Fragment Length Polymorphism) method. PCR reaction was performed in a 25 μl mixture containing 100 ng of genomic DNA, 50 mM KCl, 20 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 μM of each dNTP, 1 U Taq DNA Polymerase (Fermentas, Lithuania) and 10 pmol of each forward (5′-TAACTTCTGGACTATTTGCGGACTT-3′) and reverse (5′-GTCCAGCCCTCATCCTGGCC-3′) primers. The amplification was carried out with an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, and a final extension step at 72 °C for 5 min. After confirmation of the amplified fragment (260 bp) on agarose gel, the PCR products were digested with 1 U of MscI, Fast Digest (Fermentas, Lithuania) at 37 °C for 30 min. Digested PCR samples were electrophoresed on a 2% agarose gel at 120 V for 30 min and stained with ethidium bromide, and the genotypes were determined under UV light using a video gel documentation system (Vilber Lourmat, Cedex, France). A single 260 bp band is obtained for G nucleotide, whereas the cleaved fragment gave rise to 242 bp and 18 bp bands for T nucleotide. All three bands were observed for GT heterozygotes (Fig. 1).

2.3. Statistical analysis

Statistical analysis was performed by using PEPI 3.0 (available at: http://www.usdinc.com/pepi.html). A χ^2 analysis or Fisher's exact test was used to analyse any differences in the genotype and allele frequencies between patient and control groups. Goodness of fit χ^2 test was used to check Hardy–Weinberg equilibrium in the control population, Arlequin Software v. 2000 (University of Geneva, Geneva, Switzerland). The correlation of mean age in groups was analysed using the *t*-test for independent samples. The comparison of the categorical variables between the groups was performed by the chi-square test (SPSS 15.0, SPSS Inc., Chicago, IL, USA).

3. Results

COL1A1 Sp1 binding site polymorphism was investigated in a total of two hundred and six individuals, including one hundred and three tennis elbow patients and one hundred and three healthy control participants. Of one hundred and three patients, sixty nine (67%) had GG genotype, thirty two (31.06%) had GT and two (1.94%) had TT genotype. GG, GT and TT genotypes were found in sixty one (59.02%), thirty five (33.98%) and seven (6.8%) healthy people, respectively. There were no significant differences in genotype distribution ($p = 0.17$) and allele frequencies ($p = 0.11$) of the *COL1A1* Sp-1 binding site 1546G/T (rs1800012) polymorphism between the patient and control groups and there were no *COL1A1* genotype effects on gender, both in patients and controls ($p = 0.59$ and $p = 0.82$, respectively), on the side of the

Table 1
Demographic characteristics of patients and controls.

		Patients n = 103	Controls n = 103
Gender, n (%)	Male	30 (29.1)	22 (21.4)
	Female	73 (70.9)	81 (78.6)
Age	Years, mean (SD)	43.67 ± 9.57	41.95 ± 8.34
	Interval	21–66	26–67
Dominant hand	Right hand, n (%)	90 (87.38)	88 (85.44)
	Left hand, n (%)	11 (10.68)	12 (11.65)
	Bilateral, n (%)	2 (1.94)	3 (2.91)
Side of defect	In dominant hand, n (%)	69 (67)	NA
	In non-dominant hand, n (%)	30 (29.12)	NA
	Bilateral, n (%)	4 (3.88)	NA
Work	Heavy	29 (28.16)	NA
	Normal	74 (71.84)	NA

SD: standard deviation and NA: not assessed.

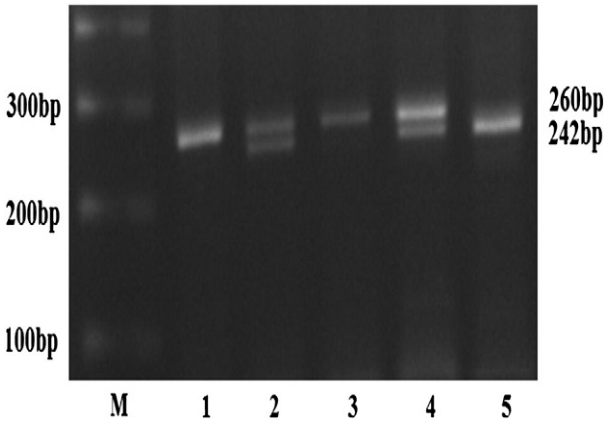

Fig. 1. A 2% agarose gel showing the genotypes of the COL1A1 Sp-1 binding site polymorphism. Lane 1: undigested sample for control, lanes 2 and 4: GT, lane 3: GG, lane 5: TT genotypes. M: 100-bp molecular weight marker (Fermentas, Lithuania).

Table 2
Genotype distributions according to the characteristics of participants.

		Total n	GG n (%)	GT n (%)	TT n (%)	p
Patients	Female	73	50 (68.5)	21 (28.8)	2 (2.7)	
n = 103	Male	30	19 (63.3)	11 (36.7)	0 (0)	
	Total	103	69 (67)	32 (31.06)	2 (1.94)	p = 0.59
Control	Female	81	48 (59.26)	28 (34.57)	5 (6.17)	
n = 103	Male	22	13 (59.1)	7 (31.8)	2 (9.1)	
	Total	103	61 (59.22)	35 (33.98)	7 (6.8)	p = 0.82
Defect in side	Right	62	41 (66.13)	19 (30.65)	2 (3.22)	p = 0.66
	Left + bilateral	41	28 (68.3)	13 (31.7)	0 (0)	
Defect in patients	In dominant hand	68	46 (67.65)	20 (29.41)	2 (2.94)	
	In non-dominant hand	30	19 (63.3)	11 (36.7)	0 (0)	
	Bilateral	4	4 (100)	0 (0)	0 (0)	p = 0.80

defect (p = 0.66) and defect being of whether dominant or non-dominant/bilateral hands (p = 0.80) (Table 2).

4. Discussion

Ligaments and tendons are collagenous structures which have very similar compositions, but different functions (Hildebrand et al., 2004). Although the pathologies of tendon and ligament injuries are not the same, some intrinsic and extrinsic risk factors are common for both, and therefore it is reasonable that they may be influenced by similar genetic factors (September et al., 2009). As well as the report suggesting the association of the guanine–thymine dinucleotide repeat polymorphism within the tenascin-C gene with Achilles tendon injury (Mokone et al., 2005), the association of *COL5A1* BstUI polymorphism with both anterior cruciate ligament rupture (Posthumus et al., 2009b) and Achilles tendinopathy (Mokone et al., 2005; September et al., 2009) was also reported. However, any specific genetic defect has not been identified as the cause of tendinopathy (Riley, 2004).

Type 1 collagen is the major protein of tendons and ligaments and is encoded by *COL1A1* gene. A nucleotide variation (1546G/T) in the first intron of the gene was reported to increase the binding affinity of Sp1 transcription factor. Therefore, the expression of *COL1A1* gene and the production of COL1α1 chain increase. However, the constituents of type I collagen: α1 and α2 chains should be in a particular ratio. The increased production of COL1α1 chain seems to impair this ratio and thus the structure of type 1 collagen protein. Mann et al. (2001) reported that the ratio of α1 chain to α2 chain in osteoblasts was 2/1 as the expected value in GG homozygote subjects while this ratio was 2,3/1 in GT heterozygote individuals. Thus, the bones derived from GT heterozygote individuals have shown reduced strength when compared to the bone strength of the subjects with GG genotype. Therefore he suggested that T variant predisposes to osteoporosis by affecting bone mass and bone quality (Mann et al., 2001).

Sp1 binding site polymorphism of the *COL1A1* gene was found to be related with cruciate ligament ruptures in Swedish and South African participants, in two different studies (Khoschnau et al., 2008; Posthumus et al., 2009a). Two reports were in accordance with each other that TT genotype was significantly underrepresented in patients when compared to control subjects. The authors suggested that rare TT genotype had a protective role against cruciate ligament ruptures. Recent data indicate that the risk associated with different polymorphisms of the same gene could be due to the necessity of studying haplotypes rather than individual variants. Accordingly, it has been shown by Ficek et al., (2013) that specific haplotypes may modify the risk of anterior cruciate ligament (ACL) injuries. Ficek et al. studied two different polymorphisms (−1997G/T and +1245G/T) of *COL1A1* gene in professional soccer players having ACL injury and suggested that COL1A1 G–T haplotype (1997G and 1245T) is associated with reduced risk of ACL rupture and may be protective against this injury (Ficek et al., 2013). Even though T nucleotide is associated with impaired type I collagen, it has not been fully understood how TT genotype would reduce the probability of having a soft tissue injury.

On the other hand, Posthumus et al. (2009c) found no association between this genetic variation and Achilles tendinopathy or ruptures of the Achilles tendon (Posthumus et al., 2009c). Consistent with this report, we could not find a relationship between lateral epicondylitis and Sp1 binding site polymorphism. In our study, 1.94% of patients and 6.8% of healthy subjects had TT genotype and there was no significant difference between the groups (p = 0.17).

The other functional polymorphisms of the intron 1, promoter region or the encoding regions of *COL1A1* gene were not investigated in this study, therefore the probability of the association of different gene variants of *COL1A1* with tennis elbow cannot be excluded.

This study has a small sample size with limited power. Additional studies including larger cohorts are required to confirm these results as well as to elucidate the biological effects of genetic variation in tennis elbow. In addition, it is highly unlikely that a single nucleotide variation may be associated with the development of the disease, since numerous proteins are involved in tendon structure, development and regeneration. These alternative possibilities need to be further investigated.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship and publication of this article.

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