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What is This?
The BstUI and DpnII Variants of the COL5A1 Gene Are Associated With Tennis Elbow

Julide Altinisik,* PhD, Gokhan Meric,† MD, Mehmet Erduran,‡ MD, Omer Ates,§ PhD, Ali Engin Ulusal,¶ MD, and Devrim Akseki,¶ MD

Investigation performed at Balikesir University Medical Faculty, Balikesir, Turkey

Background: Tennis elbow entails pain and tenderness over the lateral epicondyle. The exact cause of the condition is not fully understood. Type V collagen is a minor fibrillar collagen that intercalates with type I collagen and forms collagen fibrils. It is encoded by the COL5A1 gene. Sequence variants within COL5A1 3′-UTR have been implicated in musculoskeletal diseases.

Purpose: To determine whether rs12722 (BstUI C414T polymorphism) and rs13946 (DpnII C230T polymorphism) of the COL5A1 gene are associated with an increased risk of tennis elbow.

Study Design: Cohort study; Level of evidence, 3.

Methods: A total of 152 patients with tennis elbow and 195 healthy participants were enrolled in this study. The rs12722 (BstUI C414T) and rs13946 (DpnII C230T) polymorphisms were investigated with the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method.

Results: There was a significant difference in both BstUI and DpnII genotype frequencies between patients with tennis elbow and healthy participants. The A2 allele of BstUI and the B1 allele of DpnII were significantly underrepresented in the patient group.

Conclusion: Individuals with the BstUI A1 allele and DpnII B2 allele of the COL5A1 gene have a high likelihood of developing symptoms of the tennis elbow. This is the first study reporting that rs12722 and rs13946 SNPs (single nucleotide polymorphisms) are genetic risk factors for tennis elbow.

Keywords: tennis elbow; COL5A1 gene; BstUI polymorphism; DpnII polymorphism
COL5A1 gene mutations in about half of the cases of classic Ehlers-Danlos syndrome, characterized by joint laxity and fragility of connective tissues, emphasizes the significance of this collagen in tissue structure and function.\textsuperscript{18,19,21} Therefore, it is reasonable that sequence variants in the COL5A1 gene may increase the tendency of individuals to develop musculoskeletal soft tissue injuries. As previously reported, the BstUI variant of the COL5A1 gene was found to be associated with Achilles tendinopathy,\textsuperscript{21,24} anterior cruciate ligament (ACL) injuries in women,\textsuperscript{23} and variations in range of motion (ROM) measurements,\textsuperscript{4,6} endurance running performance,\textsuperscript{22} joint laxity,\textsuperscript{2} and tendon structures.\textsuperscript{16} Because this gene has been associated with Achilles tendinopathy and ACL ruptures, it may also predispose to other musculoskeletal soft tissue injuries, such as tennis elbow. In our previous paper,\textsuperscript{11} we investigated COL1A1 Sp1 binding site polymorphism but did not find an association with tennis elbow.

This is the first study to our knowledge examining the association of the COL5A1 gene with tennis elbow. The aim of the study was to determine whether rs12722 (BstUI C414T polymorphism) and rs13946 (DpnII C230T polymorphism) of the COL5A1 gene are associated with an increased risk of tennis elbow.

**METHODS**

A total of 154 patients (107 women and 47 men) with tennis elbow (42 patients performing repetitive activities and/or heavy work; 112 patients with sedentary lifestyle) and 195 apparently healthy participants (152 women and 43 men) without any history of tendon or ligament injury were enrolled in this study. The patients were diagnosed according to clinical criteria that have been reported previously: tenderness to palpation of the lateral epicondyle, pain with resisted wrist and middle finger extension, and activity-dependent pain around the lateral epicondyle.\textsuperscript{5,9,27} The exclusion criteria were history of trauma, fracture, and/or surgery within 12 months at the upper extremity; history of cervical disk herniation and/or radiculopathy; and symptoms of radial tunnel syndrome. The study groups did not differ in ethnicity; the patients (tennis elbow [TE] group) and the controls were all chosen from the same ethnicity/race to avoid population stratification. Our control group consisted of healthy participants chosen from the staff of the Balikesir University Medical Faculty and Balikesir University Training and Research Hospital, Turkey. The exclusion criteria for healthy subjects were a history of trauma, fracture, and/or surgery within 12 months at the upper extremity. Both the TE and control groups were interviewed by use of a questionnaire form to obtain information on demographics, personal details, and medical history. This study was approved by Gaziогmanpasa University ethics committee and conformed to the current Declaration of Helsinki guidelines.

The participants within the TE and control groups were similarly matched by age (41.36 ± 7.26 years vs 40.12 ± 6.34 years, respectively; \(P = .27\)), dominant hand (right, 87.01% vs 85.64%; respectively; \(P = .912\)), and sex. Ninety-three patients had tennis elbow in their dominant arm (60.39%), 55 had tennis elbow in their nondominant arm (35.71%), and the condition was bilateral in 6 patients (3.9%). The demographic characteristics of participants are given in Table 1.

Genomic DNA was extracted from blood samples of both patients and healthy participants by use of Gene Jet Genomic DNA Purification Kit (Fermentas) according to the manufacturer’s instructions. Polymerase chain reaction (PCR) to amplify a segment of 3’-UTR of the COL5A1 gene including BstUI and DpnII polymorphic sites was performed with 20 pmol each of forward (5’-GAGCGTTCTCGGAGATG-3’) and reverse (5’-GAAGGCACCTGCGAATAAGC-3’) primers in a PCR reaction mixture (50 μL) containing 0.2 mM of each dNTP, 20 mM Tris-HCl (pH 8.3), 1.5 mM MgCl\(_2\), 50 mM KCl, 2 U of Taq DNA Polymerase (Fermentas) and 200 ng of genomic DNA. The mixture was heated to 94°C for 5 minutes and then subjected to 35 cycles of 94°C for 1 minute, 53°C for 1 minute, and 72°C for 1 minute. The final extension was carried out for 10 minutes at 72°C. After confirmation of the amplified fragment of 667 base pairs (bp) by agarose gel, the PCR products were divided into 2 portions. The first portions of the products were digested with 1 μL of Bsh1236I (isoschizomer of BstUI), Fast Digest (Fermentas) at 37°C for 20 minutes. After digestion, the samples were electrophoresed through 2% agarose gel at 120 V for 30 minutes and stained with ethidium bromide, and the genotypes were determined under ultraviolet illumination by use of a video gel documentation system (Vilber-Lourmat). A single band of 667 bp was obtained for A3 allele; 316, 271, and 80 bp fragments for A2 allele; and 351 and 316 bp for A1 allele (Figure 1). The second portions of the PCR products were digested with 1 μL of Sau3AI (isoschizomer of DpnII), Fast Digest (Fermentas) to produce 418, 194, 40, and 15 bp fragments for B1 allele.

**TABLE 1**

<table>
<thead>
<tr>
<th>Sex, n (%)</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>47 (30.52)</td>
<td>43 (22.05)</td>
</tr>
<tr>
<td>Female</td>
<td>107 (69.48)</td>
<td>152 (77.95)</td>
</tr>
<tr>
<td>Age, y, mean ± SD (range)</td>
<td>41.36 ± 7.26 (21-55)</td>
<td>40.12 ± 6.34 (20-52)</td>
</tr>
<tr>
<td>Dominant hand, n (%)</td>
<td>Right</td>
<td>134 (87.01)</td>
</tr>
<tr>
<td>In nondominant arm</td>
<td>17 (11.04)</td>
<td>23 (11.8)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>3 (1.95)</td>
<td>5 (2.56)</td>
</tr>
<tr>
<td>Side of defect, n (%)</td>
<td>In dominant arm</td>
<td>93 (60.39)</td>
</tr>
<tr>
<td>In nondominant arm</td>
<td>55 (35.71)</td>
<td>NA</td>
</tr>
<tr>
<td>Bilateral</td>
<td>6 (3.9)</td>
<td>NA</td>
</tr>
<tr>
<td>Type of work, n (%)</td>
<td>Heavy</td>
<td>42 (27.3)</td>
</tr>
<tr>
<td>Normal</td>
<td>112 (72.7)</td>
<td>NA</td>
</tr>
</tbody>
</table>

*NA, not assessed.*

**TABLE 2**

<table>
<thead>
<tr>
<th>Type of work, n (%)</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
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</tr>
<tr>
<td>Heavy</td>
<td>42 (27.3)</td>
<td>NA</td>
</tr>
</tbody>
</table>
and 612, 40, and 15 bp fragments for B2 allele (Figure 2).
The resulting fragments were separated on 2% agarose
gel and genotyped under ultraviolet light.

Data were analyzed using the PEPI 3.0 statistical pro-
gram (http://www.brixtonhealth.com/pepi4windows.html).
The data were initially analyzed for the whole group and
then separately by sexes. A χ² analysis or Fisher exact
test was used to determine any differences in the genotype
and allele frequencies between the TE and control groups.
A goodness-of-fit χ² test using Arlequin Software v 2000
(University of Geneva) was used to check the Hardy-Wein-
berg equilibrium in the control population. The correlation
of mean age in groups was analyzed by use of the t test for
independent samples. Categorical variables were com-
pared between the groups by use of the χ² test with
SPSS 15.0 (SPSS Inc).

RESULTS

BstUI and DpnII polymorphic sites were investigated in
a total of 349 individuals, including 154 patients with ten-
sis elbow and 195 healthy control participants. There was
a significant difference in the frequencies of BstUI geno-
types (P = .029) and alleles (P = .030) between the TE
and control groups (Tables 2 and 3).

The frequency of A1 allele was higher in the TE group
(175 A1; 56.80%) compared with the control group (189
A1; 48.46%). The A2 allele frequency was higher in the con-
trol group (189 A2; 48.46%) than in the TE group (124 A2;
40.25%) (odds ratio [OR], 1.40; 95% CI, 1.04-1.89; P = .03). Of 6 genotypes of BstUI, when A1A1 genotype was
compared with other 5 genotypes, there was a significant
difference (OR, 2.01; 95% CI, 1.27-3.18; P = .003). When
we grouped subjects according to sex, we also found a differ-
ence between female TE versus control and male TE versus
control participants (P = .02 and .03, respectively) (Table 3).

There was also a significant association in genotype and
allele frequencies of DpnII between TE and control groups
and 612, 40, and 15 bp fragments for B2 allele (Figure 2).

(P = .004 and .002, respectively). The frequency of B2 allele
was higher in the TE group (133 B2; 43.10%) compared
with the control group (121 B2; 31.02%), while the B1 allele
frequency was higher in the control group (263 B1; 68.90%)
versus the TE group (175 B1; 56.80%).

DISCUSSION

One of the 2 main findings of this study is that BstUI var-
iants within the 3'-UTR of the COL5A1 gene are associated
with tennis elbow. There was a significantly higher fre-
quency of A2 allele between the control and the TE group
(48.46% vs 40.25%, respectively) and a lower frequency of
A1 allele (48.46% vs 56.8%, respectively) (OR, 1.40; 95% CI
1.04-1.89; P = .03). There were also significant differences
between the genotypes of female patients (n = 107) and con-
trols (n = 152) and between male patients (n = 47) and con-
trols (n = 43) (P = .02 and .03, respectively). As a result,
individuals with A2A2 genotype are protected against
chronic degenerative changes in tennis elbow whereas those
with A1 allele are at increased risk of developing the disease.

As collagen is the main constituent of the tendons, a var-
iation in the sequences of collagen genes may result in
a predisposition to tendinopathies. The COL5A1 gene 3'-
UTR region site is a very important site, as the genetic var-
iances in this region affect messenger RNA (mRNA) stabil-
ity and thus protein function or characteristics. There are
2 restriction sites for the BstUI restriction enzyme in the
667 bp fragment of 3'-UTR of the COL5A1 gene, which
gives 3 alleles (A1, A2, A3) when cleaved.13 One of these
sites is C414T, which means that the T variant replaces
the C variant in the 414th position. In many studies, the

Figure 1. A 2% agarose gel representing the genotypes of
BstUI polymorphism. Lanes 1 and 6, A2A2; lanes 2-4,
A1A1; lane 5, A1A2; lane 7, A1A3. M is the 100 bp molecular
weight marker (Fermentas).

Figure 2. A 2% agarose gel representing the genotypes of
DpnII polymorphism. Lanes 1 and 2, B1B2; lane 3, B2B2;
lane 4, B1B1. M is the 100 bp molecular weight marker
(Fermentas).
results have been reported as CC, CT, and TT genotypes. To provide clarity between the 2 different results, it should be stated that the A2 allele refers to the C allele.

Although COL5A1 gene polymorphisms have not been studied in tennis elbow before, different single nucleotide polymorphisms (SNPs) of this gene have been investigated in tendon and ligament injuries. South African\(^{19}\) and Australian\(^{22}\) populations with Achilles tendinopathy have been genotyped for 7 polymorphic sites. Two of them (rs3196378 and rs11103544) were located in putative mRNA binding sites. There was a significant difference in the genotype distribution of rs3196378 between Australian controls and patients, but not South African ones. Consistent with our results of rs12722 (BstUI polymorphism), 2 studies\(^{22,25}\) have reported the protective role of A2 allele (C variant). In our study, A2 was found to be a protective allele in both female and male participants, but Posthumus et al\(^{23}\) reported that only female participants with A2 allele had a decreased risk of ACL ruptures. Bell et al\(^{2}\) reported the association of A2 allele with lesser magnitudes of joint laxity. This allele has also been associated with changes in ROM, particularly with increasing age, and has been reported to be an important contributing factor to ROM variation.\(^{3}\) Kubo et al\(^{16}\) measured the mechanical properties of tendon structures in plantar flexors and knee extensors using ultrasonography and genotyped all subjects for COL5A1 gene BstUI variants. These authors reported that the maximal tendon elongation and strain of individuals with A2 allele were significantly greater than those in the individuals with other genotypes for knee extensors, but not for plantar flexors.

Our second finding is that DpnII C230T (rs13946) polymorphism is also associated with tennis elbow. B2 allele (C variant) had a higher frequency in the TE group (43.10%) than in the control group (31.02%). The frequency of B1 allele (T variant) was higher in the control group (68.9%) than in the TE group (56.80%). The differences in the genotype and allele distributions of DpnII between the control and TE groups were significant. (\(P = .004\) and \(P = .002\), respectively). Therefore, individuals with the T variant are less likely to have symptoms of tennis elbow (OR, 1.65; 95% CI, 1.21-2.26), whereas the C variant is a risk factor for the disease. In previous reports, there were no associations between DpnII polymorphism and Achilles tendinopathies, tendon ruptures, and ACL ruptures.\(^{21,23,24}\)

Tennis elbow is the first studied tendinopathy that has been related to DpnII polymorphism.

| TABLE 2 | Genotype Frequencies of the Tennis Elbow (TE) and Control Groups\(^a\) |
| --- | --- | --- | --- | --- | --- | --- |
|  | TE Group |  |  | Control Group |  |  |
|  | Females | Males | Total | Females | Males | Total |
| BstU1 |  |  |  |  |  |  |
| A1/A1 | 43 (40.18) | 19 (40.42) | 62 (40.25) | 41 (26.97) | 8 (18.60) | 49 (25.10) |
| A1/A2 | 32 (29.90) | 14 (13.08) | 46 (29.90) | 65 (42.76) | 20 (46.51) | 85 (43.58) |
| A1/A3 | 4 (3.73) | 1 (0.93) | 5 (3.25) | 5 (3.28) | 1 (2.32) | 6 (3.07) |
| A2/A2 | 24 (22.42) | 13 (12.14) | 37 (24) | 38 (25) | 12 (27.90) | 50 (25.64) |
| A2/A3 | 4 (3.73) | 0 (0) | 4 (2.59) | 2 (1.31) | 2 (4.65) | 4 (2.05) |
| A3/A3 | 0 (0) | 0 (0) | 0 (0) | 1 (0.65) | 1 (0.65) | 1 (0.51) |
| A1/A1/other | 43/64 | 19/28 | 62/92 | 41/111 | 8/35 | 49/146 |
| DpnII |  |  |  |  |  |  |
| B1/B1 | 36 (33.64) | 13 (27.65) | 49 (31.8) | 74 (48.68) | 20 (46.51) | 94 (48.2) |
| B1/B2 | 53 (49.53) | 24 (51.06) | 77 (50) | 62 (40.78) | 19 (44.18) | 81 (41.53) |
| Total | 107 | 47 | 154 | 152 | 43 | 195 |

\(^a\)All values are expressed as n (%).

| TABLE 3 | Allele Frequencies of the Tennis Elbow (TE) and Control Groups |
| --- | --- | --- | --- | --- |
| Allele | TE Group, n (%) | Control Group, n (%) |  | Odds Ratio (95% CI) |
|  |  |  |  |  |
| BstU1 |  |  |  |  |
| A1 | 175 (56.80) | 189 (48.46) | .030 | 1.40 (1.04-1.89) |
| A2 | 124 (40.25) | 189 (48.46) |  |  |
| A3 | 9 (2.92) | 12 (3.07) |  |  |
| DpnII |  |  |  |  |
| B1 | 175 (56.80) | 263 (68.90) | .002 | 1.65 (1.21-2.26) |
| B2 | 133 (43.10) | 121 (31.02) |  |  |
Type V collagen is an essential protein for life. Both copies of the COL5A1 gene are necessary for fibrillogenesis. Loss of function mutations have been related to irregular collagen fibrils and thus joint hypermobility in patients with Ehlers-Danlos syndrome. Moreover, the necessity of both copies of COL5A1 gene was illustrated in col5a1−/− and col5a1+/− mice by Wenstrup et al. Mice with null genotype die in utero, while heterozygous mice demonstrate grossly defective collagen fibril formation, reduced fibril number, and increased fibril diameter, supporting the hypothesis that type V collagen is critical for fibril nucleation. While mutations in the COL5A1 gene result mostly in individuals to develop some disorders, such as musculoskeletal soft tissue injuries. Several sequence variants within COL5A1 gene have been identified by different researchers, and all of these variants were within the 3′-UTR of the gene. Although 3′-UTR has a noncoding feature, it may affect mRNA stability and its export from the nucleus after transcription. The regulatory sequences within 3′-UTR control gene expression at the posttranscriptional level. Therefore, any mutation or single nucleotide variation within this region may alter the secondary structure of mRNA and thus protein characteristics. In situ studies have shown that the T functional form of 3′-UTR results in increased mRNA stability and therefore mRNA levels, resulting in increased α1 chain production. Collins and Posthumus proposed that individuals with TG genotype had an increased type V collagen production. This increase changes the structure and architecture of collagen fibrils, resulting in altered mechanical properties of musculoskeletal soft tissues.

The cause of tennis elbow is not fully understood, and several factors including genetic factors have been proposed. Repetitive activities and chronic overuse may not always be the cause tennis elbow. The genetic tendency of individuals to develop some disorders, such as musculoskeletal soft tissue injuries. Several sequence variants within COL5A1 gene have been identified by different researchers, and all of these variants were within the 3′-UTR of the gene. Although 3′-UTR has a noncoding feature, it may affect mRNA stability and its export from the nucleus after transcription. The regulatory sequences within 3′-UTR control gene expression at the posttranscriptional level. Therefore, any mutation or single nucleotide variation within this region may alter the secondary structure of mRNA and thus protein characteristics.

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