

## Short *aggrecan* gene repetitive alleles associated with lumbar degenerative disc disease in Turkish patients

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**ABSTRACT.** We investigated a possible association between *aggrecan* gene polymorphism and lumbar degenerative disc disease in Turkish patients. One hundred 20-30-year-old patients with or without low back pain were selected for the study. Lumbar magnetic resonance imaging was performed on all patients. The patient group had low back pain clinically and degenerative disc disease radiographically. The control group included patients with and without low back pain: all were negative radiographically for degenerative disc disease. Genomic DNA was extracted from all participants. A PCR assay was used to evaluate variable number of tandem repeat polymorphism of *aggrecan*

gene alleles to determine if there was any correlation with degenerative disc disease. Significant associations were found between short repeated alleles of the *aggrecan* gene and severe disc degeneration. A significant association was also found between short repeated alleles of the *aggrecan* gene and multilevel disc herniation as well as extrusion and sequestration types of disc herniation. In Turkish population, short repeated alleles of the *aggrecan* gene are associated with increased disc degeneration and disc herniation.

**Key words:** *Aggrecan* gene; Disc degeneration; Polymorphism; Lumbar

## INTRODUCTION

Back pain is one of the most common musculoskeletal diseases. It causes suffering and distress to patients and their families, and it affects a large number of people; the prevalence rates in a number of studies ranged from 12 to 35% (Maniadakis and Gray, 2000) and around 10% of affected individuals become chronically disabled. Back pain is strongly associated with degeneration of the intervertebral discs (Luoma et al., 2000). Disc degeneration, although in many cases asymptomatic (Boden et al., 1990), is also associated with sciatica and disc herniation. Although the etiology of disc degeneration is unknown, it has been suggested that genetic factors contribute to the development of lumbar degenerative disc disease (LDDD; Zamani and MacEwen, 1982; Simmons Jr. et al., 1996; Richardson et al., 1997). Epidemiological studies have shown that a family history of intervertebral disc herniation is a risk factor for juvenile herniation and significantly affects the probability of LDDD (Matsui et al., 1990, 1992, 1998).

Intervertebral discs contain an abundant extracellular matrix of a fibrillar collagen network and proteoglycans (Watanabe et al., 1998). Proteoglycans are responsible for the high water content and play a critical role in the load carriage function of the disc. Aggrecan is a large aggregating proteoglycan that is a functionally important component of intervertebral disc and articular cartilage (Watanabe et al., 1998). Recently, a polymorphism has been identified in the coding region of the human *aggrecan* gene. Expressed variable number of tandem repeat (VNTR) polymorphism occurs in exon 12, which codes for the chondroitin sulfate attachment domain. The various alleles of the *aggrecan* gene provide attachment of a different number of chondroitin sulfate chains to a proteoglycan core protein, thereby changing cartilage functional properties (Doerge et al., 1997; Roughley et al., 2002).

There have been very few studies of association between *aggrecan* gene VNTR polymorphism and LDDD. An association between the shorter alleles of the *aggrecan* gene and increased risk of multilevel disc degeneration in young Japanese women has been reported (Kawaguchi et al., 1999). On the other hand, in middle-aged Finish men, the *aggrecan* allele with 25 or less repeats or with 27 or more repeats was associated with reduced risk of dark nucleus pulposus. The *aggrecan* allele with 26 repeats reduced the risk of dark nucleus pulposus in this study (Solovieva et al., 2007).

To help clarify the role of this gene, we made study of the association between *aggrecan* gene polymorphism and LDDD in Turkish patients.

## MATERIAL AND METHODS

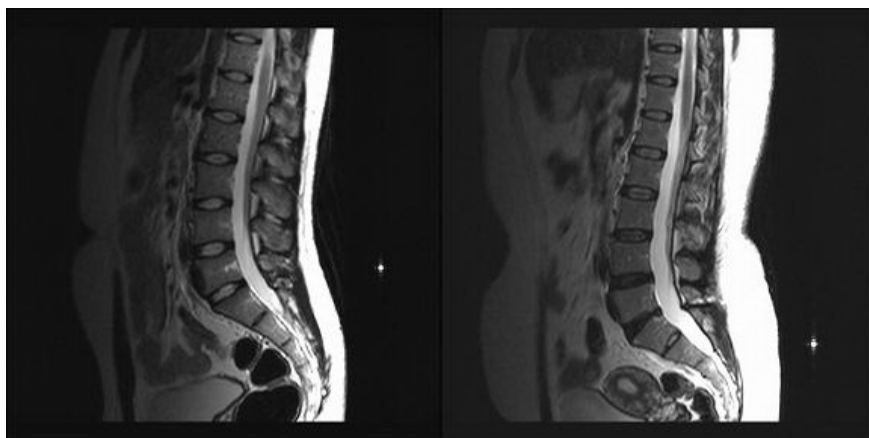
### Participants

One hundred young patients with or without low back pain and sciatica were chosen. The patient group had clinically evident low back pain, sciatica and radiographically evident degenerated discs. The control group included patients with and without clinically low back pain, but with no radiographically evident degenerated discs. None of the participants were involved in heavy physical labor or were exposed to vibration trauma, and all participants were non-smokers. The mean age of the participants was 22.3 years (range, 20-30 years). The choice of a narrow age window assures minimal age-dependent variation in disc degeneration among the individuals. Spinal stenosis and spondilolisthesis on magnetic resonance imaging (MRI) were our exclusion criteria.

This study was approved by the Human Ethics Committee of Kocatepe University (Afyonkarahisar, Turkey). Informed consent was obtained from the participants before they joined the study.

### Magnetic resonance imaging analysis

Lumbar sagittal MRI (Magnetom 1.5 T, Siemens AG, Germany) was performed with a 5-mm slice thickness. A T2-weighted image with a repetition of 2500 ms and an echo time of 90 ms of the lumbar spine was taken for all the participants. The signal intensities of the nucleus pulposus of discs L2-L3, L3-L4, L4-L5, and L5-S1 were evaluated independently by three radiologists. The grade of disc degeneration was determined according to Schneiderman's classification: grade 1, normal signal intensity; grade 2, heterogeneous decreased signal intensity; grade 3, diffuse loss of signal; grade 4, signal void (Figure 1) (Schneiderman et al., 1987). Disc herniation was evaluated with the MacNab's criterium classification (Wiesel et al., 1996): normal, protrusion, extrusion, and sequestration types (Figure 1).



**Figure 1.** Magnetic resonance image of the lumbar spine. Subject 1: a 22-year-old men with normal discs. Subject 2: a 28-year-old woman with multilevel lumbar degenerative disc disease. There was protrusion-type disc herniation at L5-S1 and L4-5.

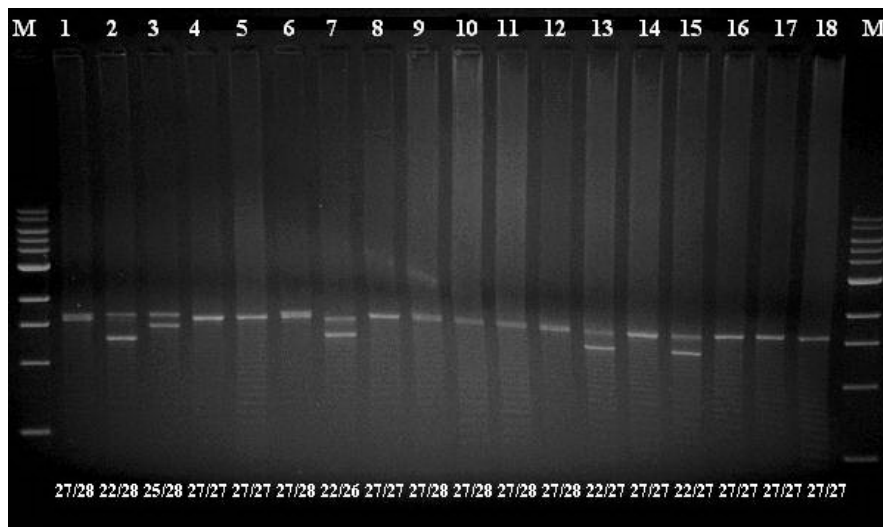
Based on MRI analysis, the levels of disc degeneration were categorized as absent, level 1, level 2, and over; the grade of disc degeneration was classified as normal, grades 1-2 (mild), grades 3-4 (severe) (Table 1). The disc herniation types were divided into three groups: normal, protrusion, extrusion, or sequestration. In addition, levels of disc herniation were categorized as absent, level 1, level 2, and over (Table 2).

### Genomic DNA analysis

Peripheral blood was collected and genomic DNA was extracted from the samples. The polymorphism of the *aggrecan* gene was detected using a polymerase chain reaction (PCR) assay and agarose gel electrophoresis (Figure 2). The sequences of sense and antisense primers for PCR were 5'-TAGAGGGCTCTGCCTCTGGAGTTG-3' and 5'-AGGTCCCCTACCGCAGAGGTAGAA-3', respectively (Kawaguchi et al., 1999). PCR was carried out in a 50- $\mu$ L solution containing 10 pmol each of the sense and antisense primers, 5  $\mu$ L genomic deoxyribonucleic acid (DNA), 25 mM diethylnitrophenyl thiophosphate (dNTP), 0.5  $\mu$ L Taq DNA polymerase, 25 mM MgCl<sub>2</sub>, and 10X PCR buffer. Amplification proceeded for 38 cycles, with denaturation at 95°C for 5 min. The annealing temperature was 66°C for 50 s, and extension was at 72°C for 50 s. The PCR products were separated on 2.5% agarose gels and visualized by ethidium bromide staining.

### Statistical analysis

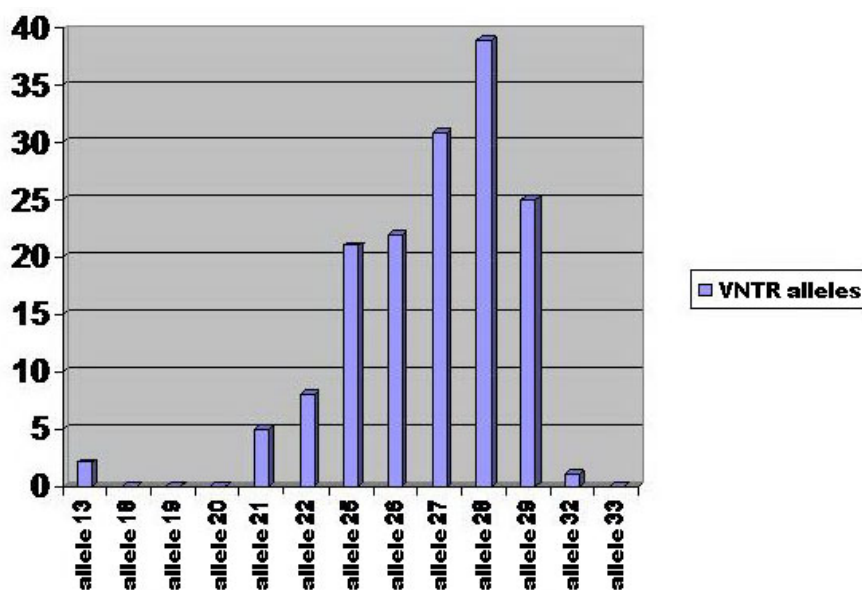
The chi-square test was performed to assess the trend of alleles regarding various parameters of disc degeneration: the number of the degenerated discs, the severity of the disc degeneration, and the number and the type of disc herniation. A P value of less than 0.05 was considered to be statistically significant.



**Figure 2.** Polymerase chain reaction analysis of *aggrecan* gene variable number of tandem repeat alleles in the subject. M = molecular weight marker.

## RESULTS

Genotyping of *aggrecan* gene VNTR polymorphism among the 100 individuals resulted in identification of nine alleles (Figure 3). There were no participants with alleles A18-20 or A33. Allele 28 was the most common form, together with alleles A27 and A29. The alleles in the participants were divided into three groups: A13-25 (shorter alleles), A26-A27 (normal alleles) and A28-A32 (longer alleles) (Doege et al., 1997; Kawaguchi et al., 1999; Solovieva et al., 2007). A significant association was found between the shorter alleles and the severe grade of disc degeneration; there was also a significant association between normal and longer alleles and normal discs (absent of disc degeneration) (Table 1). A significant association was found between shorter alleles and multilevel disc herniation. Normal and longer alleles were significantly associated with single level disc herniation (Table 2). A significant association was found between the shorter alleles and the extrusion and sequestration types of disc herniation. The distribution was significantly different between normal and longer alleles and protrusion type of disc herniation (Table 2).



**Figure 3.** Distribution of the *aggrecan* gene variable number of tandem repeat alleles among 100 young Turkish patients.

**Table 1.** Distribution of alleles of the *aggrecan* gene polymorphism with respect to the level and grade of disc degeneration.

Alleles	Level of disc degeneration			P	Grade of disc degeneration			P
	Absent	Level 1	≥2 levels		Normal	Grades 1-2	Grades 3-4	
13-25	19	5	22	0.005	19	5	22	0.005
26-27	37	10	24	<0.001	37	21	12	0.001
28-32	44	14	25	<0.001	44	29	11	<0.001

The distribution was significantly different between the two groups, with an overrepresentation of shorter alleles, which was 13-25 repeats in cases with multilevel (≥2 levels) and severe degeneration (grades 3-4) ( $\chi^2$  test for trend).

**Table 2.** Distribution of alleles of the *aggrecan* gene polymorphism with respect to the level and type of disc herniation.

Alleles	Level of disc herniation			P	Type of disc herniation			P
	Absent	Level 1	≥2 levels		Normal	Protrusion	Extrusion/sequestration	
13-25	1	11	14	0.005	1	13	14	0.004
26-27	9	19	4	0.004	9	20	3	0.001
28-32	15	21	6	0.017	15	23	2	<0.001

A significant association was found between the shorter alleles and multilevel (≥2 levels) disc herniation ( $\chi^2$  test for trend). A significant association was found between the shorter alleles and the extrusion and sequestration types of disc herniation.

## DISCUSSION

We found severe disc degeneration, multilevel disc degeneration and extrusion-sequestration type disc herniation to be associated with short alleles of the *aggrecan* gene. We did not find differences in disc herniation type and grade, or level of disc degeneration in the normal and long allele groups.

Lumbar degenerative disc disease is one of the most frequently seen spinal diseases. Age, occupations involving handling of heavy loads, motor vehicle driving, vibration, and smoking have been reported as risk factors for structural damage of intervertebral discs (Kelsey et al., 1984; Pope and Hansson, 1992; Kujala et al., 1996). Recent studies have shown an association between genetic influences and disc degeneration. To date, several gene loci associated with human disc degeneration have been identified. The identification of specific genetic influences may eventually provide key insights into underlying mechanisms. Furthermore, for specific genes and some environmental factors, gene-gene interactions and gene-environment interactions may exist (Kalichman and Hunter, 2008).

Battie et al. (2009) found 31 studies on the association of genes and disc degeneration. Among the 23 genes that have been investigated in 31 studies, 17 were associated with disc degeneration, pathological changes, or associated symptoms in at least one study. They also reported three studies about the *aggrecan* gene in different populations (Finnish, Japanese, Canadian).

Videman et al. (2009) selected 25 candidate genes for their study (*aggrecan* [AGC1], collagen [COL], interleukin [IL], and matrix metalloproteinase [MMP] genes). AGC1 was associated with disc desiccation, bulging, and height narrowing, and COL9A1 and COL9A2 were associated with disc desiccation and bulging. The COL1A1, IL1A, and IL18RAP genes were associated only with disc desiccation and COL11A1 as well as COL3A1 genes with disc bulging (Videman et al., 2009). However, no association between the *aggrecan* gene and lumbar degenerative spinal stenosis was found by Nojonen-Hietala et al. (2003). We found an association between the *aggrecan* gene and LDDD in our present study.

VNTR polymorphism in the coding region of the human *aggrecan* gene has been identified (Doege et al., 1997). A total of 14 different alleles have been observed, with repeat numbers ranging from 13 to 33 (Doege et al., 1997; Kirk et al., 2003). The frequency of the alleles found in other studies was different between populations. In the Finnish population, the frequency of the alleles was calculated in two different studies (six and nine different alleles, respectively) (Nojonen-Hietala et al., 2003; Solovieva et al., 2007). In young Japanese and Canadian populations, eight and 13 repetitive alleles were reported, respectively (Kämäräinen

et al., 2006; Roughley et al., 2006). In our study, we did not detect alleles containing 18 to 20 or 33 repeats; alleles A28, A27 and A29 (containing 28, 27 and 29 repeats, respectively) were over represented in the individuals.

There have been very few studies of *aggrecan* gene VNTR polymorphism. Kawaguchi et al. (1999) previously reported that subjects with polymorphism of the *aggrecan* gene are at risk for early disc degeneration. Multilevel and severe disc degeneration presented more often in young Japanese women with shorter (18 and 21 repeats) alleles than in those with longer (26, 27, and 28 repeats) alleles. On the other hand, no difference was found in the incidence of disc degeneration for individuals with at least one allele with less than 25 repeats by Roughley et al. (2006) and Solovieva et al. (2007). Our results are similar to what was found in the Japanese population.

There was a protective effect of the alleles with 25 repeats and longer alleles (with 27 or more repeats) on disc degeneration (Roughley et al., 2006; Solovieva et al., 2007). Similarly, other studies indicated that allele A27 provides protection from hand osteoarthritis but shorter or longer alleles may predispose subjects to this disease (Horton et al, 1998; Kämäräinen et al., 2006). We found that normal and longer alleles were associated with reduced risk of disc degeneration.

In the Japanese population, no significant association was found between the various alleles and the number or type of disc herniation (Kawaguchi et al., 1999). In contrast, we found that multilevel and extrusion and sequestration types of disc herniation were most common in patients with shorter alleles of the *aggrecan* gene.

A number of genes have been associated with intervertebral disc degeneration in humans, including genes coding for collagen I, collagen IX (COL9A2 and COL9A3), collagen XI (COL11A2), IL-1, aggrecan, vitamin D receptor, MMP-3, and CILP. For specific genes and some environmental factors, gene-gene, gene-environment and gene-age interactions may exist. Candidate-gene association studies have limitations in detecting the genetic basis of the disease because this approach relies on having predicted the correct genes on the basis of a biological hypothesis or the location of known linkage regions. Additional studies, including linkage analyses and whole genome scan studies in different populations and the whole range of ages, are required to improve our understanding of the influence of the aforementioned genes on intervertebral disk degeneration and identify novel genes (Kalichman and Hunter, 2008).

## CONCLUSION

Severe disc degeneration, multilevel disc degeneration and extrusion/sequestration type disc herniation was found to be associated with short alleles of the *aggrecan* gene. A limitation of our study was the small sample size, which reduces the power of the study to detect differences and results in a low precision of the effect estimates. A larger-scale investigation would help elucidate some of the differences found between studies.

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