

# Association of collagen I, IX and vitamin D receptor gene polymorphisms with radiological severity of intervertebral disc degeneration in Southern European Ancestor

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## Abstract

**Purpose** Several genomic loci have been previously found to be associated with intervertebral disc degeneration, so far. Data are mostly derived from northern European countries whereas data derived from Southern European Ancestor are limited. This study aimed to evaluate the association between radiological disease severity of lumbar disc degeneration and certain genetic loci in a sample of participants from Southern Europe.

**Methods** Seventy-five patients with mild to severe lumbar disc degeneration and 25 healthy controls were enrolled into the study. In each subject, each lumbar intervertebral disc was separately examined to obtain a total radiological score for disease severity. In addition, single-nucleotide polymorphisms of predefined genetic samples were analyzed in all participants: COL1A1 Sp1, COL9a2 Trp2, COL9a3 Trp3, and VDR TaqI.

**Results** Degeneration scores were significantly worse in cases with COL1A1 Sp1, COL9a3 Trp3, and VDR TaqI mutations; however, COL9a2 Trp2 mutation was not associated with a difference in the severity of disc degeneration. In addition, subjects with mutation in more than

one gene sample ( $n = 20$ ) had significantly worse degeneration scores than the remaining study participants ( $n = 80$ ) ( $17.70 \pm 2.72$  vs.  $21.81 \pm 1.81$ ,  $p < 0.001$ ).

**Conclusion** Single-nucleotide polymorphisms occurring in COL1A1, COL9a3 and VDR genes seem to be associated with the development of lumbar disc degeneration in this cohort, possibly with even more pronounced association when multiple mutations are present in the same individual. By further prospective twin studies in associated genes and analyses of their relationship with environmental factors in an internationally sampled large cohort will make a more clear-minded conclusion about their association with disc degeneration, which would yield better appreciation and clinical planning of some predisposed people for these pathologies.

**Keywords** Single-nucleotide polymorphism (SNP) · Collagen 1A1 (COL1A1) · Collagen 9 alpha-2 (COL9a2) · Collagen 9 alpha-3 (COL9a3) · Vitamin D receptor (VDR)

## Abbreviations

SNP Single-nucleotide polymorphism  
M Male  
F Female  
MRI Magnetic resonance imaging  
CT Computed tomography

## Introduction

Intervertebral disc degeneration and lumbar disc herniation are the two most common spinal conditions in adults. Low back pain represents the most important cause of work-related disability in people less than 45 years of age [1, 2].

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Degeneration in intervertebral discs begins after the first decade of life [3]. In conjunction with degeneration, the equilibrium between the breakdown and buildup within the disc is disturbed, resulting in alteration in its biomechanical properties. Also influenced by biomechanical forces, a degenerated disc is more prone to herniation associated with severe low back and extremity pain. The most common concern related with disc degeneration was based on repetitive mechanical forces on intervertebral disc material, which was found to be less likely than genetic influences [4–7]. The association between intervertebral disc degeneration and genetic mutations has originally been proposed by Videman et al. [7] and Jones et al. [8] in 1998. The genomic structures that have been found to be associated with disc degeneration in subsequent studies include the vitamin D receptor (VDR), aggrecan gene, type I and type IX collagen mutations, and the matrix metalloproteinase enzyme gene [7, 9–13].

This study aimed to evaluate the association between disease severity and certain genetic mutations implicated in the occurrence of lumbar disc degeneration in a cohort from Turkey, Southern part of Europe. For this purpose, a gene analysis was performed in different subsets of the study population including subjects with symptoms of disc degeneration and healthy controls to assess the correlation between the genetic disorder and the severity of the radiological degeneration.

## Materials and methods

### Patients

Seventy-five patients with mild to severe lumbar disc degeneration and 25 healthy controls were included in this study. Exclusion criteria included current smoking, a profession requiring heavy physical work, history of spinal trauma, inflammatory or infectious disorders of spinal column, and congenital conditions. To rule out confounding factors related to gender or age differences, all patients were selected among male subjects between 35 and 45 years of age.

The study was conducted conjointly at Departments of Neurosurgery at Marmara University, and Marmara University Institute of Neurological Sciences. All patients provided written informed consent before participation in the study. The study protocol was approved by the Ethics Committee of the Medical Faculty of Marmara University (Approval code: MAR-YC-2008-0199).

### Neuroradiological assessments

Magnetic resonance imaging (MRI) was performed in all participants for neuroradiological assessment. For this

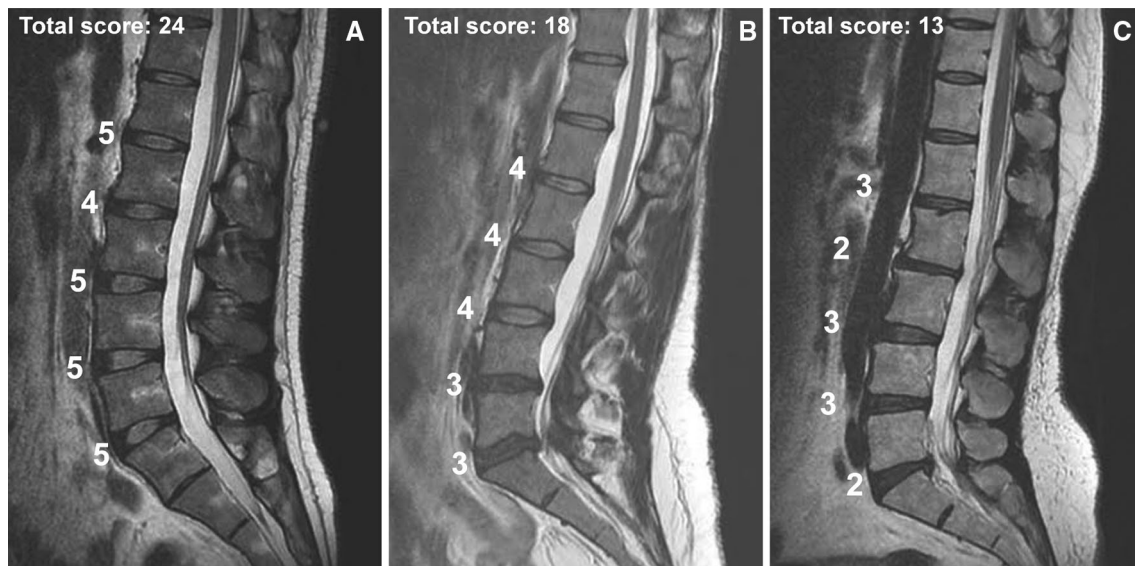
purpose, T2-weighted sagittal cross-sections were obtained to score intervertebral disc degeneration based on the classification system proposed by Pfirrmann et al. [14]. Pfirrmann's classification differentiates intervertebral disc degeneration into 5 grades. While grade 1 corresponds to healthy intervertebral disc (homogenous bright white structure), grade 5 means advanced degeneration of intervertebral disc, accordingly. A point was given for every grade in a reverse manner (5 points to grade 1 intervertebral disc, 1 point to grade 5 intervertebral disc). Each lumbar intervertebral disc was separately scored from L1 to S1 to obtain a total score (max 25, min 5). Examples of scoring are given in Fig. 1. Kappa coefficients for intra- (0.84–0.90) and interobserver (0.69–0.81) reliability were reported high for Pfirrmann's classification [14].

### Genetic analyses

For all study participants, the following single-nucleotide polymorphisms described previously for Collagen 1A1 (COL1A1), Collagen 9 alpha-2 (COL9a2), Collagen 9 alpha-3 (COL9a3), and Vitamin D receptor (VDR) genes were analyzed: COL1A1 Sp1, COL9a2 Trp2, COL9a3 Trp3, and VDR TaqI. DNA samples were extracted from venous blood samples (9 cc from each patient) in ethylenediamine tetraacetic acid (EDTA) tubes stored in cold chain. Refgen Biotechnology and Gene Research Institute (METU, Ankara, Turkey) performed all stages of the genetic analysis.

### DNA isolation

After blood samples in EDTA tubes were transferred to another tube, 35 ml of double-purified ddH<sub>2</sub>O at +4 °C was added and stirred together for 5 min. Then, the samples were centrifuged at 2000 rpm for 10 min, and the supernatant was discarded from the tubes. A total of 25 ml of water at +4 °C was added into the precipitate, stirred for 5 min with a vortex, and centrifuged at 2000 rpm for 10 min again. Supernatant was discarded, and 3 ml of nucleolysis buffer, 200 microliter 10 % SDS, and 150 microliter proteinase K (10 mg/ml) were added and the mixture was dissolved with vortex stirring. After completion of this stage, the mixture was maintained at 37 °C for one night. Then, 2 ml of ammonium acetate (9, 6 M) was added. The mixture was kept at room temperature for 10 min, centrifuged at 3000 rpm for 20 min. On that occasion, the supernatant was transferred to another tube and absolute ethanol approximately two times the volume of the supernatant was added into it. The tube was slowly stirred to allow accumulation of genomic DNA. The DNA was transferred to a tube containing 1 ml of distilled water with the tip of a pipette. The purity and concentration of the



**Fig. 1** Three examples of lumbar intervertebral disc degeneration assessment for each intervertebral space using Pfirrmann's classification [14]

resultant DNA samples were ascertained using a RNA/DNA meter (Pharmacia Biotech, New Jersey, USA).

### Polymerase chain reaction

Polymerase chain reaction (PCR) was used to amplify the genomic DNA obtained through the abovementioned procedures. For this purpose, 50  $\mu$ l of reaction volume was used that contained 100 ng of DNA, 1.25 units (U) Taq polymerase enzyme, 5  $\mu$ l of 10X PZR buffer (the concentration in reaction is 1X), 5  $\mu$ l of 2 mM dNTP, 25 pmol/ $\mu$ l of appropriate primers, and 1 mM of MgCl<sub>2</sub>. The 50  $\mu$ l of reaction volume was obtained by the addition of ddH<sub>2</sub>O. DNA was allowed to accumulate, transferred to a tube containing 1 ml of distilled water using the tip of a pipette, and dissolved. The purity and concentration of the DNA samples obtained were ascertained using a RNA/DNA meter (Pharmacia Biotech, New Jersey, USA). In all five reactions that were allowed to occur within the context of the study procedures, an initial denaturation temperature of 95 °C for 5 min, and final temperature of 72 °C for 7 min were used.

### Agarose gel electrophoresis and DNA sequence analysis

Agarose gel electrophoresis was used to observe the products of the PCR reactions. Agarose gel (1 %) was used for col1A1, Col9a2, Col9a3 and VDR regions. To obtain 1 % agarose gel, 0.25 g of agarose was weighed in a sensitive weighing scale and dissolved with the use of heat after addition of 25 ml of 0.5X TBE. After addition of ethidium bromide, the solution was poured into a gel tank. PCR products were loaded while being mixed with gel loading

buffer. The products proceeded for 15–30 min depending on the length of the band at 100 V within the gel. DNA sequence analysis was performed on PCR products. The sequence analysis was performed in accordance with the method described in the instruction manual of the “Big Dye Terminator v3.1 Cycle Sequencing” kit (Applied Biosystems, Massachusetts, USA). Following the sequence PCR, a sequence cleansing was performed and the sequence was loaded into the device (ABI 3100 genetic analyzer, Applied Biosystems, Massachusetts, USA). The sequences read were compared with the reference sequences of the genome bank.

### Statistical analyses

Statistical analyses were performed using SPSS Statistics for Windows, version 17.0. (Chicago: SPSS Inc, USA) statistics software package. Chi-square test was used to analyze significance level. The cut-off value for statistical significance was set at an alpha level of <0.05.

### Results

All participants were males at an age of between 35 and 45 years (mean age = 40.99 years). Overall Pfirrmann scores were between 10 and 25 (mean: 20.99  $\pm$  2.59). The observed associations between radiological scores and genetic variations are as follows (Table 1).

There were 32 participants with heterozygote allele and 8 participants with homozygote allele of COL1A1 Sp1. There was a significant difference in Pfirrmann scores between neutral and heterozygous cases (21.88  $\pm$  1.91 vs.

**Table 1** Gene polymorphisms found in the presented study

<i>N</i> = 100	Collagen 1A1 Sp1	Collagen 9a2 Trp2	Collagen 9a3 Trp3	Vitamin D receptor
Heterozygous	32	3	5	39
Homozygous	8	None	None	11

20.16 ± 2.81,  $p = 0.003$ ). Significant difference was present between neutral and homozygous cases (21.88 ± 1.91 vs. 17.63 ± 2.62,  $p < 0.001$ ). Hence, there was also statistically significant difference between heterozygous and homozygous cases for COL1A1 Sp1 mutations (20.16 ± 2.81 vs. 17.63 ± 2.62,  $p = 0.013$ ).

A total number of three participants were found to harbor heterozygote allele of COL9a2 Trp2 gene locus. COL9a2 Trp2 polymorphism did not differ significantly in terms of Pfirrmann scores between neutral and heterozygous cases, even though scores were lower in the heterozygous group (21.12 ± 2.36 vs. 16.67 ± 6.11,  $p = 0.119$ ). Of the three heterozygous participants, two had had previous lumbar spinal surgeries and recurrent diseases. No homozygous cases were detected for this mutation.

There were five participants with heterozygote allele of COL9a3 Trp3. Cases neutral for COL9a3 Trp3 had significantly higher Pfirrmann scores as compared to heterozygous cases (21.07 ± 2.63 vs. 19.40 ± 1.14,  $p = 0.044$ ). All of the five heterozygous cases had had previous lumbar disc surgery with recurrence in three. No homozygous mutations were observed.

There were 39 and 11 participants with heterozygote and homozygote allele of VDR TaqI, respectively. A significant difference in Pfirrmann scores was found between those with neutral, heterozygous or homozygous VDR TaqI. Scores in neutral cases were significantly higher compared to both heterozygous cases (22.15 ± 1.83 vs. 20.61 ± 2.68,  $p = 0.004$ ) and homozygous cases (22.15 ± 1.83 vs. 18.45 ± 2.51,  $p < 0.001$ ). In addition, heterozygous cases had significantly higher scores than homozygous cases (20.61 ± 2.68 vs. 18.45 ± 2.51,  $p = 0.008$ ).

Subjects with mutations in more than one gene location ( $n = 20$ ) had significantly lower Pfirrmann scores than the remaining study participants (17.70 ± 2.72 vs. 21.81 ± 1.81,  $p < 0.001$ ). Only one out of 25 healthy controls had multiple mutations (4 %). On the other hand, 19 out of 75 symptomatic patients had multiple mutations (25.3 %). The difference was significant ( $p = 0.021$ ).

## Discussion

Previous genetic studies on different populations have pointed out a propensity for intervertebral disc degeneration in individuals with SNP at certain genetic loci, which has also been the subject of the current study (Table 2) [7–11, 13,

15–48]. Genomic structures that have been associated with disc degeneration include the vitamin D receptor, aggrecan gene, type I and type IX collagen genes, and matrix metalloproteinase enzyme gene [7, 9–13]. A good knowledge of genetic factors associated with increased risk of disc degeneration bears some clinical significance, particularly in terms of predicting and preventing prevalent diseases such as lumbar disc herniation and low back pain.

In a recent and comprehensive review, approximately 20 gene locations have been detected that play a role in disc degeneration, those 20 locations were categorized into five functional groups: structural, catabolic, anticatabolic, inflammatory, other [49]. In this study, we analyzed 3 of the 20 gene locations, accordingly: Collagen I, collagen IX (a2 and a3 subtypes), and VDR.

Collagen I is the major building collagen unit of osseous tissues, annulus fibrosis, and the vertebral end-plates. In this regard, it is interesting to note that Grant et al. [50] described a polymorphism in the first intron of COL1A1 gene resulting as substitution of guanine (G) with thymidine (T) that is associated with decreased binding of a transcription factor referred to as Sp1. In that study, TT (homozygous) and GT (heterozygous) alleles have been found to represent a significant risk factor for osteoporosis. In recent years, COL1A1 Sp1 polymorphism has been frequently implicated in the development of osteoporosis and spinal degeneration [51]. The association between the genetic polymorphism of collagen I and disc degeneration was originally shown by Pluijm et al. [15]. On the other hand, Tilkeridis et al. [16] found an increased frequency of homozygote genotype in patients with lumbar disc disease. In the present study, the assessment of the association between COL1A1 Sp1 and intervertebral disc degeneration showed an increased predisposition to disc degeneration in subjects with mutations, which might be related to a change in the composition of collagen in the disc. Sp1 allele is resultant of an intronic polymorphism with potential adverse effects on transcription of collagen I.

Collagen IX, which is thought to play a bridging role between non-collagenous and collagenous tissues [10], is a heterotrimeric protein and contains three chains referred to as  $\alpha 1(\text{IX})$ ,  $\alpha 2(\text{IX})$ , and  $\alpha 3(\text{IX})$  that are separately coded by COL9a1, COL9a2 and COL9a3 genes, respectively [52, 53]. Collagen 9a2 is an SNP region that has been the subject of several studies in terms of its association with the risk of intervertebral disc degeneration [10, 11, 17–22]. For example, the sequence variations of the COL9a2

**Table 2** Previous genetic studies on different populations examining the relation between certain SNPs and intervertebral disc degeneration

Candidate protein	Genomic name	Chromosomal location	References	Origin	Gender	Number of cases	Definition of degeneration	Relation with intervertebral disc degeneration
COL1A1	Collagen I alpha 1	17q21.3-q22	Noponen-Hietala et al. [19]	Finland	M and F	29 (56 controls)	MRI, CT	Absent
			Pluijm et al. [15]	Holland	M and F	517	Plain x-ray	Present
			Tilkeridis et al. [16]	Greece	M	24 (12 controls)	MRI	Present
			Videman et al. [29]	Finland	M	588	MRI	Present
			Annunen et al. [10]	Finland	M and F	157 (174 controls)	MRI	Present
			Wrocklage et al. [17]	Germany	M and F	250	Surgery	Present
			Paasilta et al. [11]	Finland	M and F	171 (321 controls)	MRI, back pain	Absent
			Karppinen et al. [18]	Finland	M and F	159	Clinical findings, MRI	Present
			Noponen-Hietala et al. [19]	Finland	M and F	29 (56 controls)	MRI, CT	Present
			Kales et al. [26]	Greece	M and F	105 (102 controls)	Clinical findings, MRI, CT	Absent
COL9a2	Collagen IX alpha 2	1p33-p32.3	Matsui et al. [46]	United States of America	M and F	108	Imaging (not specified)	Present
			Jim et al. [20]	China	M and F	804	MRI	Present
			Seki et al. [21]	Japan	M and F	470 (658 controls)	MRI	Present
			Solovieva et al. [25]	Finland	M	135	MRI	Present
			Higashino et al. [22]	Japan	M and F	84	Surgery	Present
			Videman et al. [29]	Finland	M	588	MRI	Present
			Kelempisioti et al. [30]	Finland	M and F	538	MRI	Absent
			Rathod et al. [23]	India	M and F	100 (100 controls)	MRI, back pain	Present
			Paasilta et al. [11]	Finland	M and F	171 (321 controls)	MRI, back pain	Present
			Solovieva et al. [24, 25]	Finland	M	135	MRI	Present
COL9a3	Collagen IX alpha 3	20q13.3	Noponen-Hietala et al. [19]	Finland	M and F	29 (56 controls)	MRI, CT	Present
			Karppinen et al. [45]	Finland	M and F	153	MRI	Present
			Kales et al. [26]	Greece	M and F	105 (102 controls)	Clinical findings, MRI, CT	Absent
			Matsui et al. [46]	United States of America	M and F	108	Imaging (not specified)	Present
			Jim et al. [20]	China	M and F	804	MRI	Absent
			Higashino et al. [22]	Japan	M and F	84	Surgery	Absent
			Videman et al. [29]	Finland	M	588	MRI	Absent

**Table 2** continued

Candidate protein	Genomic name	Chromosomal location	References	Origin	Gender	Number of cases	Definition of degeneration	Relation with intervertebral disc degeneration
VDR	Vitamin D receptor	12q12-q14	Eskola et al. [31]	Denmark	M and F	66 (154 controls)	MRI	Absent
			Kelempisioti et al. [30]	Finland	M and F	538	MRI	Absent
			Rathod et al. [23]	India	M and F	100 (100 controls)	MRI, back pain	Absent
			Videman et al. [7]	Finland	M	170	MRI	Present
			Jones et al. [8]	Australia	M and F	282	Plain x-ray	Present
			Videman et al. [27]	Finland	M	142	MRI	Present
			Kawaguchi et al. [9]	Japan	M and F	205	MRI	Present
			Nojonen-Hietala et al. [19]	Finland	M and F	29 (56 controls)	MRI, CT	Absent
			Oishi et al. [32]	Japan	F	60	BMD, plain x-ray, MRI	Absent
			Jordan et al. [44]	United Kingdom	M and F	291	Plain x-ray	Present
			Valdes et al. [13]	United Kingdom	F	720	Plain x-ray	Present
			Cheung et al. [28]	China	M and F	388 (191 controls)	MRI	Present
			Koshizuka et al. [47]	Japan	F	318	Plain x-ray	Present
			Sakai et al. [33]	Japan	M and F	387	Plain x-ray	Absent
			Virtanen et al. [34]	Finland	M	150 (61 controls)	Clinical findings	Absent
			Nunes et al. [35]	Brazil	M and F	66 (88 controls)	MRI	Present
			Yuan et al. [36]	China	M and F	178 (284 controls)	CT	Present
			Eskola et al. [31]	Denmark	M and F	66 (154 controls)	MRI	Absent
			Eser et al. [37]	Turkey	N/A	150 (150 controls)	MRI	Present
			Kelempisioti et al. [30]	Finland	M and F	538	MRI	Absent
Omar et al. [38]	Norway	M and F	146 (188 controls)	Plain x-ray, MRI, CT	Absent			
Chen et al. [43]	China	M and F	81 (101 controls)	MRI	Present			
Colombini et al. [40]	Italy	M and F	267 (220 controls)	MRI	Present			
Cervin Serrano et al. [39]	Mexico	M and F	100 (100 controls)	MRI	Absent			
Vieira et al. [41]	Brazil	M and F	121 (131 controls)	MRI	Present			
Zawilla et al. [42]	Egypt	N/A	84 (60 controls)	Clinical findings, MRI	Present			
Colombini et al. [48]	Italy	M and F	267 (254 controls)	MRI	Present			

coding the  $\alpha 2$  chain of collagen IX in the intervertebral disc have been studied in a Finnish population [10]. In that study, the rare Trp2 allele, which is located at codon 326 of COL9a2 and substitutes the natural amino acid arginine with tryptophan, was found to be present in 6 of 157 patients (allele frequency 1.9 %), while none of the healthy controls ( $n = 174$ ) had this allele. Although this represents an important finding, the false negativity could not be ruled out since the study did not involve non-operated symptomatic patients. Our study is similar to that study in terms of the rare occurrence of Trp2 allele. In a study from China, 20 % of the study population has been found to have Trp2 allele, associated with a fourfold increased risk of annular rupture and 2.4-fold increased risk of disc degeneration [20]. In a Japanese study by Seki et al. [21], a specific haplotype COL9a2 has been found to be related with disc degeneration. Trp2 allele existed in 21.4 % of the patients undergoing lumbar discectomy. In a recent study, Zhu et al. examined collagen IX SNP mutations and used immunohistochemical methods to check the presence of collagen IX in the intervertebral disc space. These authors have proposed that collagen IX, which almost never exists in the normal adult disc, is produced in the context of a response to mechanical disc injury [54].

Trp3 polymorphism previously described in COL9a3 was present in five of the 100 cases in this study, who had also increased radiological severity scores compared to the remaining participants. In a Greek population, which may be considered to bear genetic similarities to Turkish population due to geographical neighborhood, Trp3 allele (COL9a3) was present in 8.6 % of those who underwent disc surgery, as compared to 4.9 % in healthy controls, with the difference being unable to reach statistical significance [26]. Solovieva et al. [24, 25] found a positive correlation between Trp3 allele and disc degeneration in 135 middle-aged men in their 2002 study, and proposed in another report published in 2006, that interleukin 1 beta gene polymorphism could modify this association. All of the patients with heterozygote allele of Trp3 in the presented study were bearing degenerated disc and had intervertebral disc disease-related problems. Three of the patients had already developed recurrent disc disease.

A study from India showed higher frequency of collagen 9a2 heterozygosity in those individuals with disc pathology, despite the absence of a similar association for collagen 9a3 [23] which is in contrast with our findings. This difference may be due to an unidentified genomic factor or another biological pathway, which was not addressed in our study; or this may be related geographical variance of the genomic material and influences of different environmental factors over genes.

Vitamin D receptor (VDR) is known to play a role in normal bone mineralization and bone remodeling.

Genomic mutations of VDR have been associated with several conditions including osteoporosis, osteoarthritis, and degenerative disc disease [27, 55, 56]. A TaqI polymorphism on this gene has previously been described, and the resultant homozygous or heterozygous genotypes have been linked with degenerative intervertebral disc conditions [28]. In an Australian cohort study involving 172 female and 110 male patients, the frequency of VDR alleles was 63 % for “T” and 37 % for “t” [8], and the “tt” allele was found to be significantly associated with osteophyte formation and radiologically documented disc degeneration. Radiologically lower signal intensity was present in 12.9 and 4.5 % of the subjects with TaqI “tt” and “Tt” genotypes, respectively, as compared to controls. Another retrospective study suggested a higher rate of annular rupture in those patients with the “tt” genotype [27]. In a Japanese population between 20 and 29 years of age, allele variations of the VDR gene have been found to be linked with severe disc degeneration at multiple levels [9]. In another study with providing more solid statistical evidence in terms of its larger sample size (804 participants), the odds ratio for disc degeneration was quite high, i.e., 2.61, for those subjects having the t allele of the VDR gene [28]. Further analyses in the same subjects showed even a more marked association for those less than 40 years of age (odds ratio 5.97). Similarly, in our study a significantly lower score for lumbar disc degeneration was observed in those subjects carrying the VDR TaqI polymorphism.

Many genetic studies, which looked for possible gene loci that may be in association with disc degeneration, have been conducted in ancestors of Northern Europe or Far East Asia. There are two previous studies conducted in Greece, two in Italy and one in Turkey. Although these five studies have already looked for all these four gene loci, they did not analyze them all together. To our knowledge, this is the first study of looking for possible association of these four genes with disc degeneration in a southern Europe country, in separate and in combination [16, 26, 37, 40, 48]. One other important point to mention is that possible associations with disc degeneration have been depicted for collagen I and vitamin D receptor, but not for Collagen IX in those previous studies. Eser et al. [37] reported significant associations of VDR and aggrecan genes with intervertebral disc degeneration and disc herniation in a 300-participant Turkish cohort. They specifically studied both TaqI and FokI polymorphisms of VDR. Our study is concordant with their study in respect of TaqI polymorphism. Even though we did not look for any effect of aggrecan genes, we studied the role of collagen I and collagen IX in intervertebral disc degeneration. Not only the aggrecan and VDR genes, but also collagen 1A1 and collagen 9a3 were found to be significantly associated with

disc degeneration. In further, this study also made us to realize a possible additive effect of multiple gene polymorphisms on disc degeneration grade and presence of multiple polymorphisms more commonly in diseased intervertebral discs (25.3 vs. 4 %). This might be through gene–gene interactions or simply breakdown of all structures in the intervertebral disc translated by representative gene loci (VDR, collagen I and IX). Further studies are in need to test this hypothesis.

All SNP studies of collagen I, collagen IX and vitamin D receptor in association with intervertebral disc degeneration are summarized in Table 2. Significant associations have been depicted for COL1A1, COL9a2, VDR, and less for COL9a3. Results are heterogenous across the studies and this can be due to differences in study designs, geographical areas and study participants (twins vs. non-twins).

One of the major limitations of our study is its sample size (i.e., 75 patients with lumbar disc degeneration and 25 controls), which is resultant of prospective nature of the study. Even though, the difference between neutral and heterozygote participants for COL9a2 was not significant, the heterozygote ones had lower Pfirrmann scores. The sample size could be the most relevant factor for this result. However, the number of participants in this study is considerably enough to make comparisons with literature and to give new insights for planning future studies. In addition, the scoring method used for assessing the severity of the disc degeneration may be criticized since Pfirrmann scoring system is not sensitive to the etiology of the disc degeneration and is qualitative in nature. Alternative scoring methods share the same disadvantage of not being sensitive to etiological factors. Despite its qualitative assessment nature, it has been shown that intra- and inter-reliability of Pfirrmann classification is high [14].

Twin studies are the most appropriate model for evaluation of genetic and environmental effects on disc degeneration. It has been demonstrated that environmental factors including smoking have tiny statistically significant effect on disc degeneration process. The most striking fact was not only the degeneration itself but also degenerative events were similar in twin brothers. However, the twin studies were limited to Northern Europe and Northern America [4, 57]. Such a twin study generalized to more other populations will enhance the knowledge about the relation of gene–gene, gene–environment in respect of intervertebral disc degeneration.

## Conclusions

Our results suggest that single-nucleotide polymorphisms occurring in COL1A1, COL9a3 and VDR genes seem to be associated with the development of lumbar disc

degeneration, with even more pronounced association when multiple mutations in same individuals are present. By further prospective twin studies in associated genes and analyses of their relationship with environmental factors in an internationally sampled large cohort will make a more clear-minded conclusion about their association with disc degeneration, which would yield better appreciation and clinical planning of some predisposed people for these pathologies.

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## Compliance with ethical standards

**Conflict of interest** All the authors declare that there is no conflict of interest.

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