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# Intervertebral disc degeneration in relation to the *COL9A3* and the *IL-1*ß gene polymorphisms

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**Abstract** Disc degeneration is a complex condition in which environmental factors and multiple genes are expected to act together to determine the degenerative phenotype. Recently associations of COL9A2 (Trp2 allele) and COL9A3 (Trp3 allele) polymorphisms with lumbar disc disease characterized by sciatica have been reported. However, it is not known whether the Trp2 or Trp3 alleles contribute to disc degeneration (DD). In this study, the association between the collagen genes polymorphisms and lumbar DD was investigated. Furthermore, the influence of the IL-1β($C^{3954}$-T) polymorphism on the association of collagen genes polymorphisms with DD was examined. Lumbar intervertebral discs of 135 middle-aged occupationally active men were evaluated with magnetic resonance imaging, using decreased signal intensity of the nucleus pulposus, disc bulges, and decreased disc height as signs of degeneration. Blood samples were analysed for the presence of COL9A3 and COL9A2 tryptophan alleles (Trp3 and Trp2 alleles). The COL11A2, COL2A1 and IL-1β($C^{3954}$-T) polymorphisms were also analysed. Multivariate logistic regression analysis allowing for occupation and body mass index showed that the carriage of the Trp3 allele in the absence of the IL-1β$T^{3954}$ allele increased the risk of dark nucleus pulposus (OR 7.0, 95% CI 1.3–38.8) and joint occurrence of degenerative changes (OR 8.0, 95% CI 1.4–44.7). There was no effect of the Trp3 allele on DD in the presence of the IL-1β$T^{3954}$ allele. The carriers of the COL11A2 minor allele had an increased risk of disc bulges (OR 2.1, 95% CI 1.0–4.2) as compared with non-carriers. The results suggest that the effect of the *COL9A3* gene polymorphism on DD might be modified by the *IL-1*β gene polymorphism.

**Keywords** Lumbar disc degeneration · Magnetic resonance imaging · COL9A3 · IL-1β

## Introduction

Degeneration of the intervertebral disc is a process that begins early in life and is a consequence of a various intrinsic and extrinsic factors as well as of normal ageing. Environmental factors appear to explain only a minor part of individual variation in pathologic changes in the disc, while the major part remains unexplained [4, 5, 34]. Epidemiological studies of twins suggest that inheritance is the largest single determinant of disc degeneration (DD) [5, 27].

Recently, an association between DD and polymorphisms in the vitamin-D receptor [37, 38], aggrecan [14], matrix metalloproteinase-3 genes [33], and interleukin 1

gene cluster [30] has been reported. However, only one gene defect responsible for intervertebral disc disease has been identified [2]. A sequence variation in the α2 chain of collagen IX (*COL9A2*) that converts a codon for glutamine to a codon for tryptophan (known as the Trp2 allele) has been shown to be associated with dominantly inherited lumbar disc disease characterized by sciatica in about 4% of Finnish patients. Subsequently the same research group showed that another tryptophan allele (Trp3 allele) in the α3 chain of collagen IX (*COL9A3*) carried a threefold risk of intervertebral disc disease [21].

Lumbar disc disease characterized by lumbar disc herniation and sciatica is affecting about 5% of all individuals [1]. It often leads to physical impairment and requires surgery. Disc degeneration is believed to be an important factor in the pathogenesis of disc herniation [3]. The prevalence of intervertebral DD in the general population varies from 36 to 93% depending on age [8]. Disc degeneration is a complex condition with environmental factors and multiple genes likely acting together to determine an overall degenerative phenotype. There has been little study of the COL9A2 and COL9A3 tryptophan polymorphisms and it is not known whether the Trp2 or Trp3 alleles contribute to disc degeneration.

Intervertebral discs contain an abundant extracellular matrix of proteoglycans and collagens [9]. The annulus fibrosus consists mainly of collagen I. The interior structure of the disc, nucleus pulposus, contains proteoglycan (50%), mainly aggrecan, and collagen II (20%), as a major cartilage collagen. It also contains a small amount of two other cartilage collagens, types IX and XI.

Recently we reported an association of functional polymorphism of the interleukin-1β gene with disc degeneration [30] and low back pain [31]. Interleukin-1 (IL-1) is a cytokine that is believed to play a critical role in the induction of enzymes that destroy proteoglycans [29]. It impedes the repair process by inhibiting the synthesis of collagen and proteoglycan. Earlier studies have demonstrated that IL-1β down-regulates *COL2A1* gene transcription in articular chondrocytes [10, 11]. Genetic variation in the *IL-1* genes can be expected to influence the association of collagen gene polymorphisms with DD.

The aims of the present study was (1) to investigate the possible association between disc degeneration and the collagen genes' (*COL2A1*, *COL9A2*, *COL9A3*, and *COL11A2*) polymorphisms among middle-aged occupationally active men and (2) to study the influence of the IL-1β(C-T$^{3954}$) polymorphism on the association of collagen genes' polymorphisms with disc degeneration.

## Materials and methods

The subjects of this study were a subgroup of a cohort of 1832 men from three occupations: machine drivers, construction carpenters and office workers. They had participated in two repeated questionnaire studies (1984 and 1987) concerning occupational effects on low back pain [25]. One hundred sixty-four men aged 40–45 years underwent MRI of the lumbar spine in 1991 and had completed a self-administered questionnaire. The ethical committee of Finnish Institute of Occupational Health approved the study proposal. An informed consent was obtained from the subjects. One hundred thirty-five (82%) of them donated a blood sample for genetic analysis in 1999.

The subjects had been in their present occupation for 26 years on average at the time of MRI. Occupational loading on the back was distinctly different in each occupational group. Forty-one of them were exposed to whole body vibration and prolonged sitting (machine drivers), 42 to dynamic physical work (construction carpenters) and 52 to sedentary work (office workers).

### Gene tests

Blood samples were analysed for the presence of arginine to tryptophan change in the COL9A3 gene (Trp3 allele) and glutamine to tryptophan change in the *COL9A2* gene (Trp2 allele). The alleles were detected by analysing polymerase chain reaction (PCR) products by conformation sensitive gel electrophoresis and sequencing [15, 22].

Single nucleotide polymorphisms (SNP) of the *COL11A2*, *COL2A1* and *IL-1*β genes were genotyped with the SNP–TRAP method [20] or by allele-specific primer extension on microarray [23] with some modifications. For SNP–TRAP genomic DNA was amplified by PCR. Genotypes were checked for Hardy–Weinberg equilibrium.

For technical reasons, not all blood samples were successfully genotyped: of the 135 samples analysed, five could not be genotyped for the COL11A2(G-A), eight could not be genotyped for the COL2A1(G-A), and six could not be genotyped for the IL-1β(C$^{3954}$-T).

### Assessment of disc degeneration

The lumbar spine of the subjects was imaged using a 0.1 T MRI unit. The following imaging protocol was used: sagittal dual-echo sequence using a 2000 ms repetition time and 25 and 86 ms echo times producing proton density-weighted gradient echo and T2-weighted spin-echo images. The slice thickness was 7 mm, field of view 410 × 410 mm$^2$ and the pixel size 1.6 × 1.6 mm$^2$. Sagittal MR images of region L2–L5 were obtained. If evaluation of the disc was not considered reliable, it was classified as ''missing''.

The signal intensity of the nucleus pulposus of the discs L2/L3–L5/S1 was visually estimated, by three independent readers who were blinded to occupation and genotype of the subjects. Cerebrospinal fluid at the corresponding disc level was used as a signal intensity reference since its validity has been proved [17, 18]. Intensity lower than that of the adjacent cerebrospinal fluid was considered a positive finding and was called dark nucleus pulposus. Interobserver agreement (weighted kappa) between each pair of radiologists ranged from 0.59 to 0.87 depending on disc level, being lowest for the L5/S1 disc. The data of the most experienced radiologist were used for the analysis.

The magnitude of the disc bulges (anterior and posterior) was measured by one of the radiologists (KL) in the middle sagittal line of the disc with the facility of the MRI device. A bulge reaching ≥3.2 mm (two pixels) beyond the outer edges of two adjacent vertebrae was considered a sign of degeneration, as it was the smallest size to be reliably measured. The intraobserver agreement rate ($n = 25$ subjects) was from 0.81 to 0.92 for posterior bulges and from 0.86 to 1.0 for anterior bulges depending on disc level, lowest for the L5/S1 disc. None of the subjects had a major asymmetric bulge or herniation.

The height of the L3/L4 and L4/L5 discs was visually classified independently by an experienced radiologists (AL) and a trained physician (ML) using a four-point scale (0 = normal, 1 = slightly decreased, 2 = distinctly decreased, and 3 = severely decreased). Interobserver agreement (weighted kappa) between the observers was 0.73 for the L3/L4 disc and 0.58 for the L4/L5 disc. The data of the radiologist were used for the analysis. None of the discs in this sample had a severely decreased disc height. This variable was dichotomised as normal (0) and decreased (1,2) disc height.

The multilevel lumbar DD was assessed as the number of discs with the same type of degenerative change (dark nucleus pulposus, bulge or decreased disc height). The findings in discs L3/L4–L4/L5 were classified into three categories: none of the discs, one disc and two discs with degenerative change.

The joint occurrence of dark nucleus pulposus, disc bulges and decreased disc height at each disc level was determined as the sum of signs: dark nucleus pulposus, disc bulges and decreased disc height. The score for each disc level ranged from 0 to 2 (0 = no degenerative changes, 1 = dark nucleus pulposus (with or without disc bulges or decreased disc height), 2 = simultaneous presence of dark nucleus pulposus, disc bulges, and decreased disc height). The summary score ranged from 0 to 4, on which 0 = no degenerative changes at any disc level, 4 = simultaneous presence of dark nucleus pulposus, and decreased disc height at two disc levels. This variable was finally categorised in three groups as normal (0), some degeneration (1–2) and extended degeneration (3–4) for the analysis.

## Questionnaire data

The data regarding individual characteristics were collected by self-administered questionnaires in 1991. The questionnaire included items on anthropometric measures and smoking status. Body mass indexes (BMI) [weight (kg) per height squared ($m^2$)] were calculated from height reported on the questionnaire and weight reported at the age of 25 years and at the time of MRI examination. Mean BMI at age of 25 years was 22.9 (SD 2.5, range 18.5–33.4) and at age of 40–45 years was 25.6 (SD 3.6, range 18.8–39.5). Job title was used as the measure of occupational load.

### *Statistical analysis*

Allele and genotype frequencies were compared between individuals with and without DD using Fisher's exact probability test or chi-square test. Carriage rates for the singular nucleotide polymorphisms (SNPs) were calculated as the proportion of individuals with at least one copy of the minor allele.

The association of DD with polymorphisms of the collagen genes was analysed using proportional odds model. The dependent variables were the number of discs with degenerative changes and the number of discs with joint occurrence of all degenerative signs. Adjusted odds ratios (OR) and their 95% confidence intervals (CI) were estimated by logistic regression analysis, controlling for occupation and BMI at the age of 40–45 years.

The composite genotypes for the collagen genes and *IL-1*β gene polymorphisms comprised the presence of the minor alleles. The effect of the single gene polymorphisms and the composite genotype on DD was estimated using logistic regression analysis. The dependent variables were the number of discs with degenerative changes and the number of discs with joint occurrence of all degenerative signs. The following dichotomous variables were included into the regression: carriage of the collagen gene minor allele only, carriage of minor allele of collagen and *IL-1*β genes. The reference group consisted of individuals without the collagen gene mutations. Occupation and BMI at the age of 40–45 years were controlled for as potential confounding factors in the analysis.

Analyses were performed with the SAS statistical software Version 8.2 (SAS Institute Inc., Cary, NC, USA).

## Results

Three (2.2%) of the persons were found to have a sequence variation in exon 19 of the *COL9A2* gene, leading to a Gln326 → Trp substitution, the Trp2 allele. All three subjects with the Trp2 allele had a dark nucleus pulposus and two of them had a bulge at one disc level. They were excluded from further analyses.

Of the remaining 132 persons, 22 (16.6%) were heterozygous for the Trp3 allele, and one (0.8%) person was homozygous for the allele. Sixteen (12.6%) of the persons were found to have a sequence variation in exon 50 of the *COL2A1* gene (G/A) that changed the amino acid Val/Ile, and 46 (35.4%) of the persons were found to have a sequence variation in intron 9 of the *COL11A2* gene (A/G). For all polymorphisms of the collagen genes the overall observed genotype frequencies were in Hardy–Weinberg equilibrium. There were no differences in the frequency of genotypes and carriage rates between the occupational groups, and overweight and non-overweight individuals.

The distribution of genotypes, allele frequencies and carriage rates of the *COL9A3*, *COL2A1*, and *COL11A2* genes in persons with and without disc degeneration are shown in Table 1. A marginal increase in the frequency of the COL11A2 minor allele was observed only for disc bulges ($p = 0.04$). The carriers of this allele had an increased risk of disc bulges (OR 2.1, 95% CI 1.0–4.2) as compared with the non-carriers. The frequency of the Trp3 and the COL11A2 minor alleles was higher but statistically non-significantly among persons with disc degeneration at both disc levels, than among those without degeneration. Two (1.6%) of the persons were carriers of all three collagen genes' minor alleles. All of them had degenerative changes at both disc levels.

Carriers of the Trp3 allele were more frequently carriers of the IL-1βT$^{3954}$ allele (0.73 vs. 0.48, $p = 0.03$). Multivariate logistic regression analysis showed that the carriage of the Trp3 allele in the absence of the IL-1βT$^{3954}$ allele significantly increased the risk of dark nucleus pulposus (OR 7.0, 95% CI 1.3–38.8) and the joint occurrence of degenerative changes (OR 8.0, 95% CI 1.4–44.7). There was no effect of the Trp3 allele on disc degeneration in the presence of the IL-1βT$^{3954}$ allele (Table 2). Logistic regression analyses showed that occupation and BMI did not affect the relation. When the analysis was repeated for the carriage of either Trp3 or COL2A1 minor allele similar results were obtained.

## Discussion

This study of middle-aged working men has shown that the association between collagen gene polymorphisms

**Table 1** Distribution of the collagen gene genotypes, alleles, and carriage rates in persons with and without disc degeneration at the L3/L4 and L4/L5 disc levels

| | Dark nucleus pulposus | | | Disc bulges | | | Decreased disc height | | | Summary score[a] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No | One | Two | No | One | Two | No | One | Two | 0 | 1–2 | 3–4 |
| COL2A1 | | | | | | | | | | | | |
| AA | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| AG | 4 | 7 | 4 | 5 | 7 | 3 | 7 | 6 | 2 | 4 | 7 | 4 |
| GG | 46 | 39 | 23 | 52 | 37 | 19 | 45 | 38 | 25 | 46 | 42 | 20 |
| A allele frequency | 0.06 | 0.08 | 0.07 | 0.06 | 0.09 | 0.07 | 0.08 | 0.07 | 0.05 | 0.06 | 0.08 | 0.08 |
| A allele carriage | 9.8 | 15.2 | 14.8 | 10.3 | 15.9 | 13.6 | 15.1 | 13.6 | 7.4 | 9.8 | 14.3 | 16.7 |
| OR (95%CI)[b] | 1.4 (0.5–3.6) | | | 1.4 (0.5–3.7) | | | 0.6 (0.2–1.6) | | | 1.4 (0.5–3.9) | | |
| COL11A2 | | | | | | | | | | | | |
| AA | 2 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 |
| AG | 14 | 18 | 12 | 16 | 16 | 12 | 18 | 14 | 12 | 14 | 19 | 11 |
| GG | 37 | 29 | 15 | 43 | 27 | 11 | 37 | 39 | 15 | 37 | 31 | 13 |
| A allele frequency | 0.17 | 0.19 | 0.22 | 0.15 | 0.21 | 0.26 | 0.17 | 0.18 | 0.23 | 0.17 | 0.19 | 0.23 |
| A allele carriage | 30.2 | 38.3 | 44.4 | 28.3 | 38.6 | 52.2 | 33.9 | 34.1 | 44.4 | 30.2 | 38.0 | 45.8 |
| OR (95%CI) | 1.6 (0.8–3.1) | | | 2.1 (1.0–4.2) | | | 1.3 (0.6–2.5) | | | 1.6 (0.8–3.2) | | |
| COL9A3 | | | | | | | | | | | | |
| Carrier of the Trp3[c] | 8 | 9 | 6 | 10 | 8 | 5 | 10 | 7 | 6 | 8 | 9 | 6 |
| Non-carrier of the Trp3 | 47 | 39 | 23 | 53 | 37 | 19 | 48 | 38 | 23 | 47 | 42 | 20 |
| Trp3 allele frequency | 0.07 | 0.09 | 0.12 | 0.08 | 0.09 | 0.13 | 0.09 | 0.08 | 0.12 | 0.07 | 0.09 | 0.13 |
| Trp3 allele carriage | 14.5 | 18.8 | 20.7 | 15.9 | 17.8 | 20.8 | 17.2 | 15.5 | 20.7 | 14.5 | 17.6 | 23.1 |
| OR (95%CI) | 1.4 (0.6–3.3) | | | 1.3 (0.5–3.0) | | | 1.1 (0.5–2.6) | | | 1.5 (0.7–3.5) | | |

Disc degeneration is assessed as number of the disc with degenerative signs

[a]Joint occurrence of the dark nucleus pulposus, disc bulges and decreased disc height

[b]Proportional odds model. Odds ratios (*OR*) and their 95% confidence intervals (*95% CI*) adjusted for occupation and BMI at the age of 40–45 years

[c]Only one individual was homozygous for the Trp3 allele, this person had all degenerative changes at both disc levels

**Table 2** Association between disc degeneration at the L3/L4 and L4/L5 disc levels and the carriage of Trp3, COL2A1 (A) and the IL-1βT$^{3954}$ alleles

| | Dark nucleus pulposus | | Disc bulges | | Decreased disc height | | Summary score | |
|---|---|---|---|---|---|---|---|---|
| | OR | 95% CI | OR | 95% CI | OR | 95% CI | OR | 95% CI |
| Absence of the Trp3 allele | 1.0 | | 1.0 | | 1.0 | | 1.0 | |
| Carriage of the Trp3 allele in the absence of the IL-1βT$^{3954}$ | 7.0 | 1.3–38.8 | 1.4 | 0.3–6.9 | 4.3 | 0.8–21.6 | 8.0 | 1.4–44.7 |
| Carriage of both alleles | 0.8 | 0.3–2.2 | 1.1 | 0.4–2.9 | 0.5 | 0.2–1.5 | 0.9 | 0.3–2.4 |
| Absence of the Trp3 and COL2A1 minor allele | 1.0 | | 1.0 | | 1.0 | | 1.0 | |
| Carriage of either the Trp3 or COL2A1 minor allele in the absence of the IL-1βT$^{3954}$ | 3.5 | 1.0–11.5 | 0.7 | 0.2–2.5 | 1.9 | 0.6–6.1 | 3.9 | 1.2–13.0 |
| Carriage of either the Trp3 or COL2A1 minor allele and the IL-1βT$^{3954}$ allele | 0.9 | 0.4–2.1 | 1.3 | 0.5–3.1 | 0.5 | 0.2–1.2 | 0.9 | 0.4–2.2 |

Disc degeneration is assessed as the number of discs with degenerative signs. Proportional odds model. Odds ratios (*OR*) and their 95% confidence intervals (*CI*) are adjusted for occupation and BMI at the age of 40–45 years

and disc degeneration of the lumbar spine is modified or negatively confounded by the IL-1β(C$^{3954}$-T) polymorphism. The association of the Trp3 allele with disc degeneration assessed as the number of discs with dark nucleus pulposus and as joint occurrence of degenerative signs was statistically significant in the absence of the IL-1βT$^{3954}$ allele. The common *IL-1*β gene polymorphism studied (IL-1βT$^{3954}$ allele) has been shown to be associated with increased IL-1 levels [24]. A recent study found that IL-1β(C$^{3954}$-T) polymorphism also influences the degree of systemic inflammation, as reflected by C-reactive protein and fibrinogen levels [7].

In this study, the frequency of the Trp3 allele was marginally increased, being 12.0%, among the persons with disc degeneration at two disc levels as compared with 7% in those without disc degeneration. Paassilta et al. [22] found that the frequency of the allele was 12.2% among patients with LDD characterised by sciatica and 4.7% among the controls extracted from the Finnish population. In addition, the COL11A2 minor allele was overrepresented among the individuals with disc bulges. The functional role of the *COL11A2* gene polymorphism in intron 9 is not known. Therefore, further studies are required to confirm this finding.

Disc degeneration has proved to be a difficult entity to study; the initiation and sequence of disc degeneration is not well understood. The classification of the degenerative changes and, especially, the definition of disc degeneration vary from one study to another. Different combinations of decreased signal intensity on T2-weighted MRI, disc height narrowing, disc bulging, endplate irregularities and osteophytes have been used [16, 21, 37]. In the current study decreased signal intensity of the nucleus pulposus, disc bulges, and decreased disc height were used as signs of disc degeneration. Signal intensity of the disc is known to correlate with degree of degeneration [39]. Most of the discs with a bulge of the size of 2.5 mm or more have been found to have a radial tear [40]. Disc height decrease has been commonly used as sign of disc degeneration, although its validity as a sign of early degeneration is questionable [19].

Recently the association between the Trp3 allele and disc degeneration defined as decreased signal intensity of the nucleus and decreased disc height among patients with sciatica has been reported [13]. We found that the carriers of the Trp3 allele were at a marginally increased risk of darkness of the nucleus pulposus and joint occurrence of degenerative changes after controlling for occupation and BMI at the age of 40–45 years. This association became statistically significant only among non-carriers of the IL-1βT$^{3954}$ allele. Previously we reported an association between the *IL-1* gene cluster functional polymorphisms and disc bulges [30]. The carriers of the IL-1βT$^{3954}$ allele had a marginally decreased risk of darkness of the nucleus pulposus and decreased disc height. The findings of the current study suggest that the IL-1β(C$^{3954}$-T) polymorphism might be either an effect modifier or a negative confounder of the relationship between *COL9A3* gene polymorphism and disc degeneration.

In complex conditions, the genotype-radiologic phenotype association might be biased by the presence of another genetic or environmental factor. The possibility that the IL-1β(C$^{3954}$-T) polymorphism is a negative confounder cannot be excluded. An increase in signal intensity of the disc can be observed in inflammatory disc degeneration [32]. If the effect of the Trp3 allele on disc degeneration is modified by the IL-1β(C$^{3954}$-T) polymorphism, producing negative interaction of these factors, this may suggest that these two factors are sufficient causes of DD with a single complementary cause (third factor) [26]. Therefore, the observed interaction might be an indirect form of antagonism, where the

presence of the Trp3 allele and the presence of the IL-1βT$^{3954}$ allele are competing factors for a single pool of susceptible individuals, those who are exposed to the third factor.

Because of the low prevalence of the Trp2 allele in the studied population and relatively small sample size, it was not possible to analyse statistically the association between the *COL9A2* gene polymorphism and disc degeneration. Yet all three persons with this genetic variant had decreased signal intensity and two of them had a posterior bulge. These findings are consistent with the results of a recent study of patients with sciatica who had the Trp2 allele [12]. The researchers found that patients with the Trp2 allele more often tended to have a radial tear in non-herniated discs.

Battié et al. [6] suggested that genetic effects are more difficult to detect in middle-aged than in young populations because of cumulative effects caused by mechanical loading. Thus we may have underestimated the effect of the Trp3 allele on lumbar disc degeneration.

Lumbar MR images were obtained from disc levels L2/L3 through L5/S1, only the L3/L4 and L4/L5 discs were analysed in the current study in order to have sufficient number of individuals with degenerative changes. Several studies reported that disc degeneration has been found to be the most advanced in L3/L4–L5/S1 levels [28, 35, 36]. The reliability of MRI assessments at the L3/L4 and L4/L5 discs was higher at those levels than at the L2/L3 and L5/S1disc levels, and many observations in L2/L3 and L5/S1 discs were classified as missing.

Due to the small number of cases, our data are too scarce to allow any conclusive results. Further studies are needed to explore the interrelation between collagen and interleukin gene polymorphisms.

## Conclusions

The results suggest that the Trp3 allele plays a minor role in overall disc degeneration. The effect of the *COL9A3* gene polymorphism on disc degeneration might be modified by the *IL-1*β gene polymorphism. Since the intervertebral disc is composed of several different types of collagens and proteoglycans, it is likely that other genes besides collagen IX genes play a more important role in disc degeneration.

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