Int J Clin Exp Med 2016;9(5):8852-8857
www.ijcem.com /ISSN:1940-5901/IJCEM0021077

Original Article

Relationship between the exon 19 polymorphism of the COL9A2 gene and lumbar disc degeneration in a Chinese population

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Received December 3, 2015; Accepted March 2, 2016; Epub May 15, 2016; Published May 30, 2016

Abstract: It is estimated that at least 30% of the population has some degree of vertebral disc degeneration by the time they reach 40 years of age, and a finding of disc degeneration is often considered normal in patients over the age of 60. Despite the frequency at which disc degeneration occurs and its often painful and debilitating consequences, the exact cause of disc degeneration remains unclear. To investigate the relationship between the exon 19 polymorphism of the COL9A2 gene and lumbar disc degeneration in a Chinese population, 239 patients were enrolled in this study: 119 patients with lumbar disc degeneration and 120 without intervertebral disc disease. All study participants were patients in our hospital between July 2013 and May 2015. Genomic DNA was isolated from all subjects, and COL9A2 genotypes were determined by polymerase chain reaction (PCR) and direct DNA sequencing in a case-control study. Statistical analysis was performed using a chi-square test and Fisher's exact test. Gln326 replacement with Trp was observed in 6 patients in the case group and 13 patients in the control group. The difference in the prevalence of Gln326 replacement to Trp between the case group and the control group was not statistically significant ($P > 0.05$). Gln326 replacement with Arg was observed in 72 patients in the case group and 64 patients in the control group. The difference in the prevalence of Gln326 replacement to Arg between the case group and the control group was not statistically significant ($P > 0.05$). MRI analysis of patients with lumbar disc degeneration showed no association between Arg326, Gln326, or Trp326 and the location of disc degeneration or the number of degenerated segments (both $P > 0.05$). In brief, the data do not support an association between exon 19 polymorphism of the COL9A2 gene and lumbar disc degeneration in this Han Chinese population.

Keywords: Lumbar disc degeneration, collagen XI, polymorphism

Introduction

Degeneration of the lumbar intervertebral disc is a general term for the accumulated biological changes that occur in the lumbar intervertebral disc tissue over a patient's lifetime and for a variety of reasons. Degeneration causes changes in the mechanical properties of the disc so that the adjacent bones and joints experience corresponding degenerative changes. The overall results may include spinal instability and even compression of nerve roots, the spinal cord, and vertebral arteries. These changes cause a corresponding syndrome of clinical symptoms and signs, and serve as the pathological basis for most spinal column diseases [1]. The pathogenesis of vertebral disc degeneration is not entirely clear, but with the progress of research in genetics and molecular biology it has been found that specific genes are associated with some intervertebral disc lesions. Solovieva, et al., indicated that single nucleotide polymorphism (SNP) of interleukin-1 had an association with the degeneration of intervertebral discs [2]. Battié, et al., found that genetic factors had a significant effect on intervertebral disc degeneration, suggesting that heredity may explain as much as 74% of the variance in disc degeneration in adult populations [3]. Other genes associated with intervertebral disc degeneration include type XI collagen AI (COLI IAI), matrix metalloproteinase-3 (MMP3), MMP9, aggrecan 1 (AGCI), cartilage intermediate layer protein (CILP), and thrombospondin (THBS2) [4-9].

Olmarker, et al., confirmed that the COL9A2 gene was closely associated with degenerative disease of intervertebral discs [10]. The relationship between the Gln326Trp polymorphism of the COL9A2 gene and intervertebral disc degeneration was reported by Hirose, et al., who noted that the prevalence of the polymorphism of specific genes varies in different regions and ethnic groups [11]. The current study investigated the relationship between the Gln326 polymorphism of the COL9A2 gene of Chinese Han people and the degenerative lesions of lumbar intervertebral discs.

Subjects and methods

Study subjects

The case group was made up of 119 patients with a clinical diagnosis of lumbar disc hernia who received inpatient treatment at the Department of Spine Surgery of the Second People's Hospital of Liaocheng City from July 2013 to May 2015. Among them, 95 were men (79.8%) and 24 were women (20.2%). The mean age was 32.5 ± 8.2 years. The control group was made up of 120 patients with non-lumbar disc hernia. Among them, 92 were men (76.7%) and 28 were women (23.3%). The mean age was 34.1 ± 7.9 years. For all members of the case group, clinical examination and magnetic resonance imaging (MRI) confirmed the diagnosis of lumbar disc hernia, and imaging verified that the Pfirrmann's grade was over grade III. All patients in the control group were volunteers with other diseases without obvious degeneration of the lumbar intervertebral discs visible on MRI. The patients in both the case group and the control group were free from chronic diseases such as hypertension and diabetes mellitus as well as other genetic diseases. There was no significant difference between the case group and the control group in terms of the patients' sex and age ($P > 0.05$), and there was a comparability between the two groups. This study was approved by the Ethics Committee of the hospital and obtained the informed consent of all subjects.

Method

Main instruments and reagents: PCR Master Mix (Fermentas Corporation), Tris saturated phenol (PolePolar Biotechnology Co., LTD.), cell lysis buffer (0.5% SDS, 3 mmol EDTA, 0.1 mmol NaCl, 0.05 mol Tris, pH 8.0), agarose, spectrophotometer (Germany Eppendorf), thermal cycler (Germany Eppendorf), electrophoresis apparatus, low-temperature high-speed centrifuge, etc. Gene sequencing and primer synthesis were completed by the Beijing Microread Gene Technology Co., Ltd.

Magnetic resonance imaging (MRI) evaluation: Patients underwent lumbar vertebral MRI scan (slice thickness of 4 mm; GE 1.5 T, Milwaukee), sagittal T2WI (echo time of 98-110 ms and repetition time of 2640-2800 ms). Results were analyzed by radiologists unrelated to this study. An evaluation was performed according to Pfirrmann's intervertebral disc degeneration grading system [12]. The degeneration grading of discs L1/2-L5/S1 as well as the position and grade of the most degenerative segments were recorded.

Extraction of genomic DNA: Blood samples (2 ml) were collected from each patient, combined with sodium citrate, and stored at -80°C. DNA was extracted by combining 300 μl of blood with 800 μl of lysis buffer and incubating at 37°C for 4 hours. Next, 500 μl of Tris-saturated phenol was added and the samples were centrifuged at 10,000 rpm for 20 minutes. The supernatant was extracted and combined with an equal volume of 1:1 phenol-chloroform. Following chloroform extraction, 1 ml of dimethyl carbinol was added to precipitate the DNA, and samples were centrifuged for 10 min at 12,000 rpm. The supernatant was discarded and the precipitate was washed with 75% ethanol. The precipitates were dried, and then suspended in 40 μl of DNAse-free water. DNA was quantified with an ultraviolet spectrophotometer and stored at -30°C.

Amplification and sequencing of target segments

Premier 5 software was used for primer design. The sequence of the downstream primer was 5'-CAAGA GGTGG TGATT GAGCA AGAGC-3'. The sequence of the upstream primer was 5'-TGGAT CTCAG TTTCC CTACCTG-3'. For DNA amplification 4 mL template was combined with 25 mL of 2x mix PCR buffer, 1.5 mL of the upstream and downstream primers (10 pmol/L) and sterile deionized water to a total volume of 50 mL. The PCR reaction procedure was as follows: pre-degeneration at 94°C for 5 minutes,

Table 1. Analysis of the collagen IXA2 gene for sequence variations

Groups	Gln326	Arg326	Trp326
Patient group (n=119)	41	72	6
Control group (n=120)	43	64	13
χ^2		0.355*	1.854#
P		0.551*	0.173#

Note: *means Gln326 *vs.* Arg326, #means Gln326 *vs.* Trp326.

Table 2. Analysis of Pfirrmann's grade in patients with lumbar disc degeneration

	Grade 3	Grade 4	*P**
Gln326 (n=41)	9	32	0.250
Arg326 (n=72)	26	46	
Trp326 (n=6)	1	5	

Note: *Fisher's exact test.

Table 3. Analysis for location of intervertebral disc degeneration in patients with lumbar disc degeneration

	L4/5	L5/S1	*P**
Gln326 (n=30)	10	20	0.560
Arg326 (n=62)	27	35	
Trp326 (n=4)	2	2	

Note: *Fisher's exact test.

Table 4. Analysis of the number of degenerated intervertebral discs in patients with lumbar disc degeneration

	Single	Multiple	*P**
Gln326 (n=41)	30	11	0.134
Arg326 (n=72)	62	10	
Trp326 (n=6)	4	2	

Note: *Fisher's exact test.

annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds. After a total of 40 cycles a final extension step was performed at 72°C for 10 min. Agarose gel electrophoresis (1%) was used to observe the PCR products. Bidirectional sequencing of PCR amplified fragments was performed using an ABI3730XL gene sequencing instrument. Results were compared with the reference sequences in NCBI using ContigExpress software.

Statistical analysis

Epidata 3.1 was used to input data and establish the database by means of the double data entry method. A logical check of the data was performed, and SAS 9.2 was used for data processing. Statistical methods include: X^2 test and Fisher's exact probabilistic method. Statistical significance was set at $P < 0.05$.

Results

COL9A2 gene sequencing analysis

Gene polymorphism analysis on the 19th exon of the COL9A2 chain of all subjects found that the replacement from Gln326 to Trp was present in 6 patients in the case group and 13 patients in the control group. The difference between the case group and the control group in terms of the replacement frequency from Gln326 to Trp was of no statistical significance ($P > 0.05$). The replacement from Gln326 to Arg was present in 72 patients in the case group and 64 patients in the control group. The difference between the case group and the control group in terms of the frequency of replacement from Gln326 to Arg was of no statistical significance ($P > 0.05$). See **Table 1**.

Analysis of the relationship of Arg326, Gln326, and Trp326 with the degree of lumbar intervertebral disc degeneration

MRI analysis was performed on the lumbar spine of all patients in the case group. There were no significant differences in degree of degeneration, position of degenerative segments, or the number of degenerative segments among patients with Arg, Gln, or Trp at position 326 in the COL9A2 gene ($P > 0.05$). See **Tables 2**-**4**; **Figure 1**.

Discussion

The function of human intervertebral discs includes buffering stresses from all directions during normal activity [13]. Degeneration of intervertebral discs can begin at a relatively early age. Patients can experience intervertebral disc degeneration as early as age 20, thereby inducing pathological changes such as herniated discs and zygapophysial joint degeneration. These changes can lead to clinical degenerative changes such as vertebral canal stenosis and protrusion of the intervertebral disc [14]. When attempting to identify the factors that induce degenerative changes in intervertebral discs, current studies frequently focus on gene polymorphisms. Many of the can-

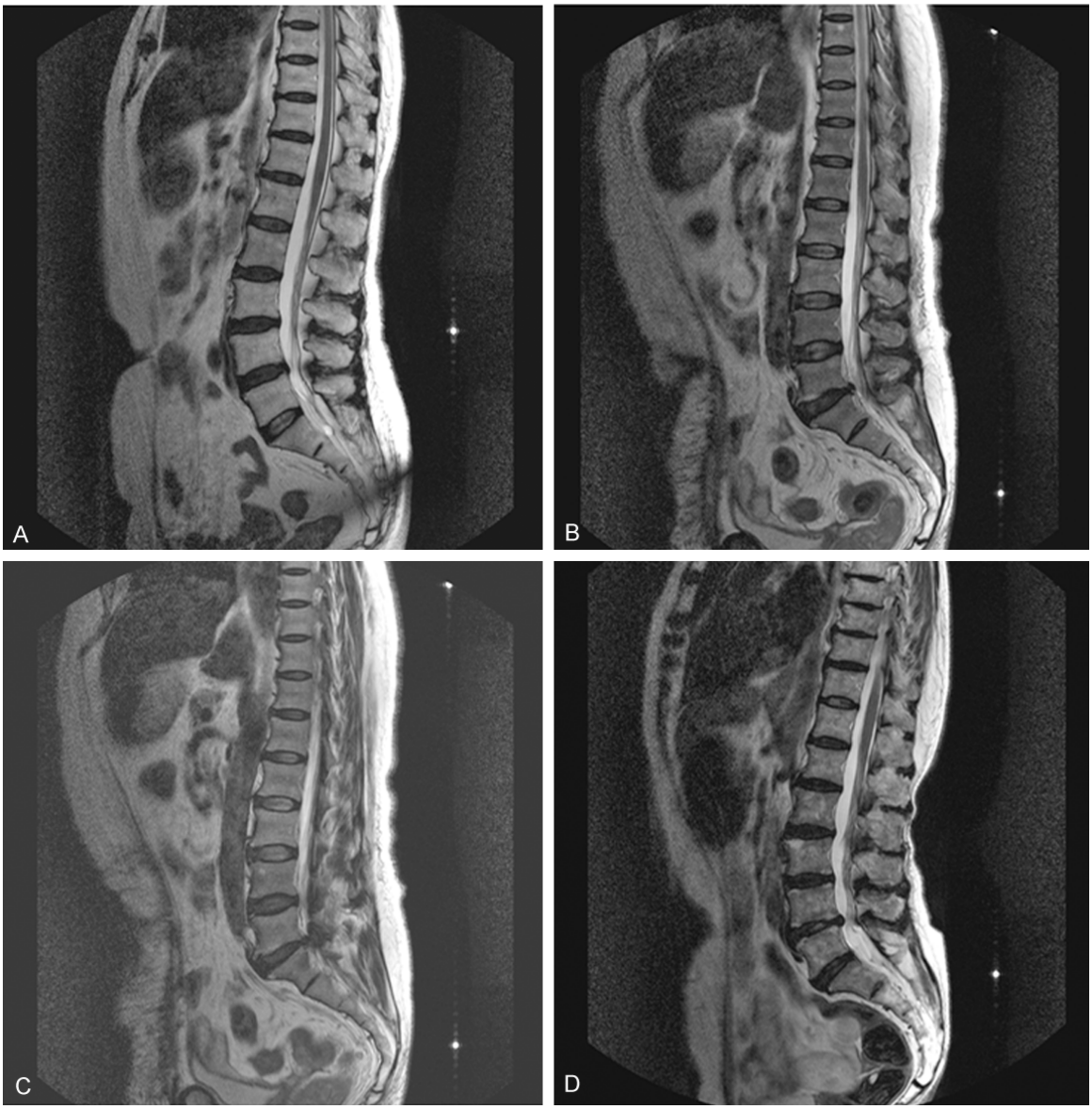

Figure 1. Diagram of the relation of Gln326, Trp326 and Arg326 with lumbar intervertebral disc degeneration MRI. A. Normal control group (grade I-II, L4/L5), a 47-year old man; B. Case group with intervertebral disc degeneration, Gln326 of 19th exon of COL9A2 chain (grade IV, L5/S1), a 35-year old man; C. Case group with intervertebral disc degeneration, Arg326 of 19th exon of COL9A2 chain (grade IV, L5/S1), a 33-year old man; D. Case group with intervertebral disc degeneration, Trp326 of 19th exon of COL9A2 chain (grade IV, L4/L5), a 33-year old man.

didate genes for degenerative diseases of lumbar intervertebral discs are genes related to the structure, composition, and metabolism of the discs.

One candidate, IX collagen, is encoded by the COL9A1, COL9A2, and COL9A3 genes, and is a heterotrimer consisting of 3 chains. The synthesis and cellular source of type IX collagen in human intervertebral discs are similar to those seen in articular cartilage, suggesting that collagen type IX in human intervertebral discs has similar functions. In the cases of intervertebral disc degeneration, the expression of the type IX collagen gene decreases or stops. This induces decreased stability between proteoglycan and type II collagen. The adhesion capacity between the collagen filaments within the pulpiform nucleus decreases, thereby undermining the biomechanical integrity of the pulpiform nucleus and leading to the degeneration of the disc [15]. Seki, et al, indicated that COL9A2 haplo-

type was associated with serious intervertebral disc diseases. They suggest that the differences in the distribution of genetic susceptibility among different regions and different races were of statistical significance [16]. The great differences among different regions and different races in terms of COL9A2 gene polymorphism increase the difficulty of studying this possibility. This study was a case control study, in which the relationship between the polymorphism of Gln326 in the COL9A2 gene of Chinese Han people and the degenerative lesions of lumbar intervertebral discs were observed.

The study found that the 19th exon of the COL9A2 chain had Gln326Arg and Gln326Trp polymorphisms. The difference between the case group and the control group in terms of the Gln326Arg and Gln326Trp polymorphism was of no statistical significance ($P > 0.05$). This suggested that, among the Chinese Han people, the polymorphism of the 19th exon of COL9A2 chain might be unrelated to the degenerative lesions of lumbar intervertebral discs. The results of the studies carried out by Knoeringer, et al, were similar: a study carried of 288 German patients who underwent surgery for protrusion of lumbar intervertebral discs indicated that COL9A2 allelic gene was unrelated with lumbar intervertebral disc degeneration among the German population [17]. In the present study, further analysis on the case group indicated that the Gln326Arg and Gln326Trp polymorphism of the 19th exon of COL9A2 were unrelated to the degree of lumbar intervertebral disc degeneration, position of degenerative segments, and quantity of degenerative segments.

Higashino, et al, indicated that adults under the age of 40 with COL9A2 allelic gene Trp2 were more apt to experience degeneration of lumbar intervertebral discs when compared with the normal control group [18]. The results indicated that there was a difference among difference races in terms of the predisposing genes of degeneration of lumbar intervertebral discs, and the genes predisposing the Chinese patients to the degeneration of lumbar intervertebral discs are unique. In the meantime, due to the different genetic backgrounds of the Chinese population in different regions such as the different frequencies of some allelic genes among the difference ethnic groups and Han groups in North China and South China, the characteristics and nature of a certain allelic gene may be affected.

In conclusion, among the Chinese Han people, the polymorphism of the 19th exon of COL9A2 chain might be unrelated with the degenerative lesions of lumbar intervertebral discs.

Disclosure of conflict of interest

None.

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References

[1] Deyo RA,Weinstein JN. Low back pain. N Engl J Med 2001; 344: 363-370.

[2] Solovieva S, Leino-Arjas P, Saarela J, Luoma K, Raininko R, Riihimäki H. Possible association of interleukin 1 gene locus polymorphisms with low back pain. Pain 2004; 109: 8-19.

[3] Battié MC, Videman T, Parent E. Lumbar disc degeneration: epidemiology and genetic influences. Spine 2004; 29: 2679-2690.

[4] Mio F, Chiba K, Hirose Y, Kawaguchi Y, Mikami Y, Oya T, Mori M, Kamata M, Matsumoto M, Ozaki K, Tanaka T, Takahashi A, Kubo T, Kimura T, Toyama Y, Ikegawa S. A functional polymorphism in COL11A1, which encodes the α1 chain of type XI collagen, is associated with susceptibility to lumbar disc herniation. Am J Hum Genet 2007; 81: 1271-1277.

[5] Kalichman L, Hunter DJ. The genetics of intervertebral disc degeneration. Associated genes. Joint Bone Spine 2008; 75: 388-396.

[6] Jackson A, Yao H, Brown MD, Yong Gu W. Anisotropic ion diffusivity in intervertebral disc: an electrical conductivity approach. Spine (Phila Pa 1976) 2006; 31: 2783-2789.

[7] Sun ZM, Miao L, Zhang YG, Ming L. Association between the-1562 C/T polymorphism of matrix metalloproteinase-9 gene and lumbar disc disease in the young adult population in North China. Connect Tissue Res 2009; 50: 181-185.

[8] Seki S, Kawaguchi Y, Chiba K, Mikami Y, Kizawa H, Oya T, Mio F, Mori M, Miyamoto Y, Masuda I, Tsunoda T, Kamata M, Kubo T, Toyama Y, Kimura T, Nakamura Y, Ikegawa S. A functional SNP in CILP, encoding cartilage intermediate layer protein, is associated with susceptibility to lumbar disc disease. Nat Genet 2005; 37: 607-612.

[9] Hirose Y, Chiba K, Karasugi T, Nakajima M, Kawaguchi Y, Mikami Y, Furuichi T, Mio F,

Miyake A, Miyamoto T, Ozaki K, Takahashi A, Mizuta H, Kubo T, Kimura T, Tanaka T, Toyama Y, Ikegawa S. A functional polymorphism in THBS2 that affects alternative splicing and MMP binding is associated with lumbar-disc herniation. Am J Hum Genet 2008; 82: 1122-1129.

[10] Olmarker K, Rydevik B. Selective inhibition of tumor necrosis factor-α prevents nucleus pulposus-induced thrombus formation, intraneural edema, and reduction of nerve conduction velocity: possible implications for future pharmacologic treatment strategies of sciatica. Spine 2001; 26: 863-869.

[11] Hirose Y, Chiba K, Karasugi T, Nakajima M, Kawaguchi Y, Mikami Y, Furuichi T, Mio F, Miyake A, Miyamoto T, Ozaki K, Takahashi A, Mizuta H, Kubo T, Kimura T, Tanaka T, Toyama Y, Ikegawa S. A functional polymorphism in THBS2 that affects alternative splicing and MMP binding is associated with lumbar-disc herniation. Am J Hum Genet 2008; 82: 1122-1129.

[12] Antoniou J, Demers CN, Beaudoin G, Goswami T, Mwale F, Aebi M, Alini M. Apparent diffusion coefficient of intervertebral discs related to matrix composition and integrity. Magn Reson Imaging 2004; 22: 963-972.

[13] Roughley PJ. Biology of intervertebral disc aging and degeneration: involvement of the extracellular matrix. Spine 2004; 29: 2691-2699.

[14] Ganey T, Hutton WC, Moseley T, Hedrick M, Meisel HJ. Intervertebral disc repair using adipose tissue-derived stem and regenerative cells: experiments in a canine model. Spine 2009; 34: 2297-2304.

[15] Solovieva S, Lohiniva J, Leino-Arjas P, Raininko R, Luoma K, Ala-Kokko L, Riihimäki H. COL9A3 gene polymorphism and obesity in intervertebral disc degeneration of the lumbar spine: evidence of gene-environment interaction. Spine 2002; 27: 2691-2696.

[16] Seki S, Kawaguchi Y, Mori M, Chiba K, Mikami Y, Tsunoda T, Kubo T, Toyama Y, Kimura T, Ikegawa S. Association study of COL9A2 with lumbar disc disease in the Japanese population. J Hum Genet 2006; 51: 1063-1067.

[17] Knoeringer M, Reinke A, Trappe AE, Schlegel J. Absence of the mutated Trp2 allele but a common polymorphism of the COL9A2 collagen gene is associated with early recurrence after lumbar discectomy in a German population. Eur Spine J 2008; 17: 463-467.

[18] Higashino K, Matsui Y, Yagi S, Takata Y, Goto T, Sakai T, Katoh S, Yasui N. The alpha2 type IX collagen tryptophan polymorphism is associated with the severity of disc degeneration in younger patients with herniated nucleus pulposus of the lumbar spine. Int Orthop 2007; 31: 107-111.