Association between *COL9A2* Gln326Arg mutations and the development of intervertebral disc disease in a Chinese population

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ABSTRACT. Intervertebral disc disease is a multifactorial condition, yet disease pathogenesis that can be promoted by a single dominant mutation affecting the expression of susceptibility genes. We performed a case-control study to assess the influence of the *COL9A2* Gln326Arg polymorphism on risk of intervertebral disc disease in a Chinese population. Between March 2014 and March 2015, a total of 215 patients and 230 healthy controls were recruited from Binzhou Medical University Hospital. Genotyping of *COL9A2* Gln326Arg was carried out using polymerase chain reaction-restriction fragment length polymorphism. Univariate and multivariate logistic regression analyses revealed that the Arg/Arg genotype of *COL9A2* Gln326Arg was associated with increased risk of intervertebral disc disease in comparison to the Gln/Gln genotype [crude odds ratio (OR) = 2.25,

95% confidence interval (CI) = 1.12-4.62; adjusted OR = 2.46, 95%CI = 1.20-5.29]. Moreover, the Arg/Arg genotype correlated with an elevated risk of this disease compared to the Gln/Gln + Gln/Arg genotypes (crude OR = 2.21, 95%CI = 1.17-4.30; adjusted OR = 2.42, 95%CI = 1.28-5.51). In conclusion, our results suggest that the *COL9A2* Gln326Arg polymorphism contributes to the development of intervertebral disc disease in the Chinese population.

Key words: Intervertebral disc disease; *COL9A2* Gln326Arg; Polymorphism

INTRODUCTION

Intervertebral disc disease involves a continuous degenerative process, the clinical features of which include reduced stability of intervertebral discs, disc herniation, degenerative scoliosis, and pain in the neck, waist, and legs (Malik et al., 2013; Izzo et al., 2015). Many factors contribute to the development of intervertebral disc disease, including long-term high- and low-pressure loads (Castagnera et al., 1991; Claus et al., 2008). In a recent study incorporating 86 monozygotic and binovular twins, the heritability of this condition was found to be 74% (Sambrook et al., 1999). To date, many investigations have indicated that vitamin D receptor, matrix metalloproteinases, and interleukins contribute to the development of intervertebral disc disease (Noponen-Hietala et al., 2005; Virtanen et al., 2007; Karli et al., 2014; Aras et al., 2016; Shi et al., 2016). However, its molecular pathogenesis is not fully understood, and the identification of novel drug targets is essential.

Collagen type IX is an important protein in joints, intervertebral discs, and the ocular vitreous body. This molecule is a heterogeneous trimer, consisting of three collagen and four non-collagen chains. The former are encoded by *COL9A1*, *COL9A2*, and *COL9A3* (Ala-Kokko, 2002). Experimental studies have indicated significant associations between collagen type IX and spinal and articular diseases (Matsui et al., 2004; Matsui, 2006; Hyun et al., 2011). It has been reported that mice homozygous or heterozygous null for *COL9A2* exhibit articular cartilage degeneration (Brachvogel et al., 2013). As yet, the role of *COL9A2* polymorphisms in the development of intervertebral disc disease in the Chinese population has not been reported. Therefore, we performed a case-control study to assess the influence of the *COL9A2* Gln326Arg variant on the risk of this disease among Chinese individuals.

MATERIAL AND METHODS

Subjects

Between March 2014 and March 2015, a total of 215 patients with intervertebral disc disease were recruited from Binzhou Medical University Hospital. Patients had complete clinical data and imaging records, and diagnoses were confirmed by X-ray and magnetic resonance imaging. Patients with a history of spinal trauma, spinal deformity, metabolic bone disease, spinal infection, or tumors were excluded.

A total of 230 healthy controls were recruited from outpatient clinics of Binzhou Medical University Hospital and using medical records during the same time period. All

control subjects were confirmed to be without discogenic pain, and had no history of lumbar trauma, metabolic bone disease, spinal infection, or malignant tumors.

A signed informed consent form was obtained from each subject prior to participation. The Ethics Committee of Binzhou Medical University Hospital authorized the performance of our study, which was conducted in accordance with the Declaration of Helsinki.

Genotyping

A peripheral venous blood sample (5 mL) was taken from each participant and stored in a tube containing 0.5 mg/mL ethylenediaminetetraacetic acid. Genomic DNA was extracted from the collected samples with a QIAamp DNA Blood Kit (QIAGEN Co., Ltd., Suzhou, China). Genotyping of *COL9A2* Gln326Arg was carried out using polymerase chain reaction-restriction fragment length polymorphism. Primers targeting the *COL9A2* Gln326Arg region were designed using Primer Premier 5.0 (PREMIER Biosoft, Palo Alto, CA, USA), and consisted of the following sequences: 5'-TGG ATC TCA GTT TCC CTA CCT G-3' and 5'-CAA GAG GTG GTG ATT GAG CAA GAG C-3'. The PCR cycling conditions used to amplify the polymorphic site were as follows: initial denaturation at 94°C for 5 min, then 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, and extension at 72°C for 45 s, before a final extension at 72°C for 10 min. PCR products were subjected to 1% agarose gel electrophoresis, and the resulting DNA bands were visualized under ultraviolet light.

Statistical methods

SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used for all data analysis. Differences in baseline and clinical variables between intervertebral disc disease patients and healthy controls were analyzed with the chi-square test. Deviation of *COL9A2* Gln326Arg genotype frequencies from Hardy-Weinberg equilibrium (HWE) was evaluated by the goodness-of-fit chi-square test. The minor allele frequency of *COL9A2* Gln326Arg was analyzed by comparing it to that in the National Center for Biotechnology Information dbSNP database (https://www.ncbi.nlm.nih.gov/snp). Univariate and multivariate logistic regression analyses were used to evaluate the association between *COL9A2* Gln326Arg and risk of intervertebral disc disease, with the results being reported as odds ratios (ORs) and 95% confidence intervals (CIs). P values less than 0.05 were considered statistically significant.

RESULTS

The demographic and clinical characteristics of the patients and controls are summarized in Table 1. The mean ages of patients with intervertebral disc disease and control subjects were 45.30 ± 9.55 and 43.45 ± 10.12 years, respectively. Compared with the controls, patients tended to have a history of intervertebral disc disease (chi-square = 9.41, P = 0.002) and be older (t = 1.98, P = 0.02). However, no significant differences were observed between the two groups in regard to gender (chi-square = 2.60, P = 0.11), body mass index (chi-square = 1.05, P = 0.31), smoking habit (chi-square = 0.11, P = 0.74), drinking habit (chi-square = 0.22, P = 0.64), manual labor (chi-square = 1.70, P = 0.19), or lumbar injury (chi-square = 2.18, P = 0.14). Ninety (41.86%) patients were at Schneiderman stages 0-I and 125 (58.14%) were at stages II-III.

Table 1. Demographic and clinical characteristics of patients and controls.

Variable	Patients (N = 215)	%	Controls (N = 230)	%	Chi-square	P
Gender						
Male	134	62.33	126	54.78		
Female	81	37.67	104	45.22	2.60	0.11
Age (years)		45.30 ± 9.55		43.45 ± 10.12	1.98	0.02
BMI						
<24	117	54.42	114	49.57		
≥24	98	45.58	116	50.43	1.05	0.31
Smoking						
No	123	57.21	128	55.65		
Yes	92	42.79	102	44.35	0.11	0.74
Drinking						
No	147	68.37	162	70.43		
Yes	68	31.63	68	29.57	0.22	0.64
Manual labor						
No	85	39.53	105	45.65		
Yes	130	60.47	125	54.35	1.70	0.19
Family history of intervertebral disc disease						
No	138	64.19	178	77.39		
Yes	77	35.81	52	22.61	9.41	0.002
Lumbar injury						
No	201	93.49	222	96.52		
Yes	14	6.51	8	3.48	2.18	0.14
Schneiderman stage						
0-I	90	41.86				
II-III	125	58.14				

BMI = body mass index.

COL9A2 Gln326Arg genotype distributions are shown in Table 2. Of the patients, 68 (31.63%), 113 (52.56%), and 34 (15.81%) carried the Gln/Gln, Gln/Arg, and Arg/Arg genotypes, respectively. Among the controls, these genotypes were observed in 81 (35.22%), 131 (56.96%), and 18 (7.82%) individuals, respectively. There was a significant difference in *COL9A2* Gln326Arg genotype distribution between patients and controls (chi-square = 6.89, P = 0.03). Using the chi-square test, the distribution of *COL9A2* Gln326Arg genotypes was found to be consistent with HWE in the control group (chi-square = 1.31, P = 0.25).

Table 2. Distribution of *COL9A2* Gln326Arg genotypes among patients with intervertebral disc disease and controls.

COL9A2 Gln326Arg	Patients (N = 215)	%	Controls (N = 230)	%	MAF	Chi-square	P	Chi-square (HWE)	P (HWE)
Gln/Gln	68	31.63	81	35.22					
Gln/Arg	113	52.56	131	56.96					
Arg/Arg	34	15.81	18	7.82	0.363	6.89	0.03	1.31	0.25

MAF = minor allele frequency, HWE = Hardy-Weinberg equilibrium.

Univariate and multivariate logistic regression analyses revealed that the Arg/Arg genotype was associated with an increased risk of intervertebral disc disease in comparison to the Gln/Gln genotype (crude OR = 2.25, 95%CI = 1.12-4.62; adjusted OR = 2.46, 95%CI = 1.20-5.29; Table 3). Furthermore, the Arg/Arg genotype of *COL9A2* Gln326Arg correlated with elevated intervertebral disc disease risk when compared to the Gln/Gln + Gln/Arg genotypes (crude OR = 2.21, 95%CI = 1.17-4.30; adjusted OR = 2.42, 95%CI = 1.28-5.51).

Table 3. Association between *COL9A2* Gln326Arg and risk of intervertebral disc disease.

COL9A2 Gln326Arg	Patients (N = 215)	%	Controls (N = 230)	%	Crude OR (95%CI)	P	Adjusted OR (95%CI)	P
Co-dominant								
Gln/Gln	68	31.63	81	35.22	1.0 (Ref.)	-	1.0 (Ref.)	-
Gln/Arg	113	52.56	131	56.96	1.03 (0.67-1.58)	0.89	1.05 (0.57-1.46)	0.72
Arg/Arg	34	15.81	18	7.82	2.25 (1.12-4.62)	0.01	2.46 (1.20-5.29)	0.01
Dominant								
Gln/Gln	68	31.63	81	35.22	1.0 (Ref.)	-	1.0 (Ref.)	-
Gln/Arg + Arg/Arg	147	68.37	149	64.78	1.18 (0.78-1.78)	0.42	1.25 (0.83-2.08)	0.38
Recessive								
Gln/Gln + Gln/Arg	181	84.19	212	92.17	1.0 (Ref.)	-	1.0 (Ref.)	-
Arg/Arg	34	15.81	18	7.82	2.21 (1.17-4.30)	0.01	2.42 (1.28-5.51)	0.001

[1]Adjusted for gender, age, and family history of intervertebral disc disease. OR = odds ratio, CI = confidence interval, Ref. = reference.

DISCUSSION

This study investigated the relationship between the *COL9A2* Gln326Arg polymorphism and development of intervertebral disc disease in a Chinese population, finding that the Arg/Arg genotype was associated with increased risk of this disease.

Previous investigations have indicated that gravitational force and occupational loading are the main risk factors for intervertebral disc disease. However, the authors of a twin study concluded that such variables in fact play a very small role in the pathogenesis of this condition (Battié et al., 1995). The gene *COL9A2* encodes an α-chain of type IX collagen, and its expression is low or absent in patients with intervertebral disc disease. Such reduced or absent expression may destabilize the interaction between proteoglycan and type II collagen, change fiber diameter, and decrease adhesion between collagen fibers in the nucleus pulposus, the biomechanical integrity of which may be compromised (Wrocklage et al., 2000; Solovieva et al., 2006; Zhu et al., 2011).

To date, several studies have tested the association between the *COL9A2* gene and development of intervertebral disc disease in various populations (Annunen et al., 1999; Kales et al., 2004; Virtanen et al., 2007; Rathod et al., 2012; Janeczko et al., 2014). Annunen et al. (1999) carried out an investigation involving 157 patients and 174 controls, identifying a *COL9A2* genetic mutation that cosegregates with the disease phenotype. Kales et al. (2004) reported that a particular polymorphism of *COL9A2* is likely to be a less significant susceptibility factor for the development of intervertebral disc disease in Greek and Finnish populations. Moreover, Rathod et al. (2012) revealed that *COL9A2* gene variation is a cause of this disease in the Indian population. However, Janeczko et al. (2014) found no association between *COL9A2* polymorphism and risk of intervertebral disc disease. In the present study, we found a significant correlation between *COL9A2* sequence variation and risk of this condition in a Chinese population. Further research is greatly needed to confirm our findings.

Our study design had two major limitations. First, the participants were selected from only one hospital, and therefore may not be representative of all patients with intervertebral disc disease and healthy individuals in the general Chinese population. Thus, selection bias was unavoidable. Second, other genes may contribute to the development of this condition, and gene-gene interaction should be considered in future study. Therefore, further studies with larger sample sizes are required to verify our conclusions.

In summary, our results suggest that the *COL9A2* Gln326Arg polymorphism contributes to the development of intervertebral disc disease in the Chinese population, although further research is needed to confirm our results.

Conflicts of interest

The authors declare no conflict of interest.

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