

Detection of *COL9A2* Gene Association with Lumbar Disc Prolapse in North Indian Population

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ABSTRACT

Inter-vertebral disc prolapse is a severe condition in the human population, so far 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: Body Mass Index, Fibrinogen, Lipid profile, Obesity.

INTRODUCTION

Lumbar disc prolapsed is a common spinal disease in adults and develops after the 2-3 decade of life. [1] It may be due to the regular breakdown of tissue within the disc by which biomechanical compositions get changed. [2] Due to this reasons many complication may occur such as disc herniation, degenerative scoliosis, and soreness in the neck, waist, and legs. A degenerated disc is having the symptoms of herniation, by which severe lower back pain and unilateral leg pain arises. [3] In the literature it is clearly mentioned that 20% of

patients with lumbar disc prolapse are needed surgical treatment during prolonged leg pain. [4]

In the lumbar disc prolapsed the effects of reduplicative mechanical forces on the inter-vertebral disc material, experimental to participate a smaller role in the disease than genetic influences. [3] There are several genetic factors found to be connected with lumbar disc prolapsed in slitrature studies include aggrecan, [5] interleukins, [6-8] vitamin-D receptor, [9] the matrix metalloproteinase, [10-11] and type I, IX, and XI collagen mutations. [12-14]

Lumbar disc prolapsed contain a abundant extracellular matrix collected of collagens and proteoglycans. Collagen IX has been measured to provide as a connecting material between collagenous and non-collagenous tissues. [15]

In the literature it has mentioned that COL9A2 gene is playing a big role in the lumbar disc prolapsed disease. Besides this many factors have been associated to the development of inter-vertebral disc disease, including long-term high- and low-pressure loads. [16] Such as in many reports it have been exposed that vitamin D receptor, matrix metallo-proteinases, and interleukins participated to the expansion of inter-vertebral disc disease. [17-18]

The study of COL9A2 gene polymorphisms would be helpful for the knowing the exact cause of lumbar disc prolapsed at gene level. [19] The present study aim was to perform COLA2 gene level studies on the genetic association of with other risk factors. This study results may offer a more accurate approximation of the relevance of these polymorphisms to lumbar disc prolapsed disease because it is an important health issue which affects both individuals and society at large. This study has its own importance since molecular pathogenesis is not fully known till now, and the identification of novel drug targets is essential. [20]

Till date, the role of COL9A2 polymorphisms in the expansion of inter-vertebral disc disease in the North Indian population has not been reported. Therefore, we conducted a case-control study to evaluate the influence of the COL9A2 on the risk of this disease among North Indian population. [21]

MATERIALS AND METHODS

After approval from the ethical committee of Rama Medical College, valid Informed consent was taken from each of the patient/subject in writing after explaining the procedure to the subject prior to entering the study.

Study type: case control study

Sample size: 200 (100 cases + 100 control)

Sample size will be calculated using this formula

$$n=4pq/(L^2)$$

p= prevalence in %

q= 1-p

L= Allowable error (10%)

After calculation we have agreed to analysis 100 cases and 100 control this study.

Cases: Inclusion criteria:

- i) Age group 18-60 years
- ii) Occupational not involving rigorous activity
- iii) Pain score of >3 score of VAS
- iv) Failed conservative management for a period of at least 3 months
- v) MRI sequences with evidences of disc prolapse /extrusion/sequestration

Exclusion criteria

- i) Age > 60 years
- ii) Occupational like manual laborers lifting heavy weights or persons dealing with vibratory tools
- iii) Body mass index (BMI) more than30
- iv) Smokers

Criteria for control:

Age matched volunteers, without any history of back pain/other symptoms without surgical history for disc prolapse

Diagnosis of disc prolapse in cases:

The cases will be examined clinically as per attached proforma [Annexure II] and all required investigations will be carried out after explaining the procedure.

Blood sampling and DNA analysis:

- * Blood samples (2ml in EDTA vial) will be collected from all the subjects
- * DNA isolation will be done and genomic DNA will be processed for PCR

The calculated minimum sample size for control group was 100.

In order to control loss of follow up and manual errors, we finalised the sample size 100 for each group.

After calculation we have agreed to analysis 100 cases and 100 control this study.

Fresh venous blood (2ml) was composed to isolate DNA by using Qiagen kit with standard protocol. Purity of DNA was estimated on 1% agarose gel. Primers oligos were got synthesized from Chromous Biotech Pvt. Ltd, Bengaluru. The obtained DNA was amplified with PCR (Bio-RAD, T-100) and the amplified DNA was resolved with 1.2% agarose gel containing ethidium bromide. Gel photographs were taken in gel documentation system (Bio-RAD).

Statistical analyses were conducted using SPSS version 22 (SPSS, USA). Statistical power of the study was predictable under log additive model, assuming 10% population risk by Quanto.^[22] Hardy-Weinberg equilibrium for genotypes was predictable by χ^2 analysis. Risk factors were estimated by logistic regression analysis assuming log-additive model. A P-value of <0.01 ($\alpha=0.05/5$) was measured significant. Mean and standard deviation were calculated in inverse normal units of the parameters in the tables. For the quantitative traits involvement, P-value of <0.00053 ($\alpha=0.05/(5 \times 19)$) was predictable to be highly significant. Association with other and quantitative traits was calculated only in control subjects. Meta-analysis of stage 1 and 2 results was performed by mixing summary data of two study population both under predetermined and random models. Likewise, the summary data of previous studies on Indians for association with Lumbar disc prolapsed^[23-24] and this study

were combined for case study. Association of variants with arthritis, back pain and quantitative traits were also conducted by mixing the data for two study population and adjusting for study population. The odds ratio (OR) and 95% confidence interval (CI) were estimated in the study.

RESULTS

Total 100 cases and 100 controls were considered for this study Between March 2018 and January 2019. Samples were belonging to the North Indian population. Magnetic resonance imaging (MRI) was conducted for diagnosis for each patient. Patients with a history of spinal trauma, spinal deformity, metabolic bone disease, spinal infection, or tumors were excluded in this study. For the controls were confirmed to be without discogenic pain, and had no history of lumbar trauma, metabolic bone disease, spinal infection, or malignant tumors.

Isolation of gene:

A venous blood sample (2mL) was taken from cases and controls and stored in deep refrigerator tube containing EDTA. Genomic DNA was isolated from the collected samples with a Qiagen DNA mini Blood Kit. Isolated DNA was amplified with PCR. The gene COL9A2 Gln326Arg was amplified using polymerase chain reaction. Primers targeting the COL9A2 Gln326Arg region were designed as mentioned in the method and materials section consisted of the following sequences.

S.N.	Primers sequences	Tm (°C)
1.	F5'-TGGATCTCAGTTCCCTACCTG-3'	55.9
2.	R5'-CAAGAGGTGGTGATTGAGCAAGAGC-3'	55.9

The PCR cycling conditions were conducted to amplify the gene were as follows: initial denaturation at 94°C for 4 min, then 38 cycles of denaturation at 94°C for 30s, annealing at 54°C for 45s, and extension at 72°C for 45s, final extension at 72°C for 7 min. PCR products were resolved with 1% agarose gel electrophoresis, and the resulting DNA bands were visualized under ultraviolet light.

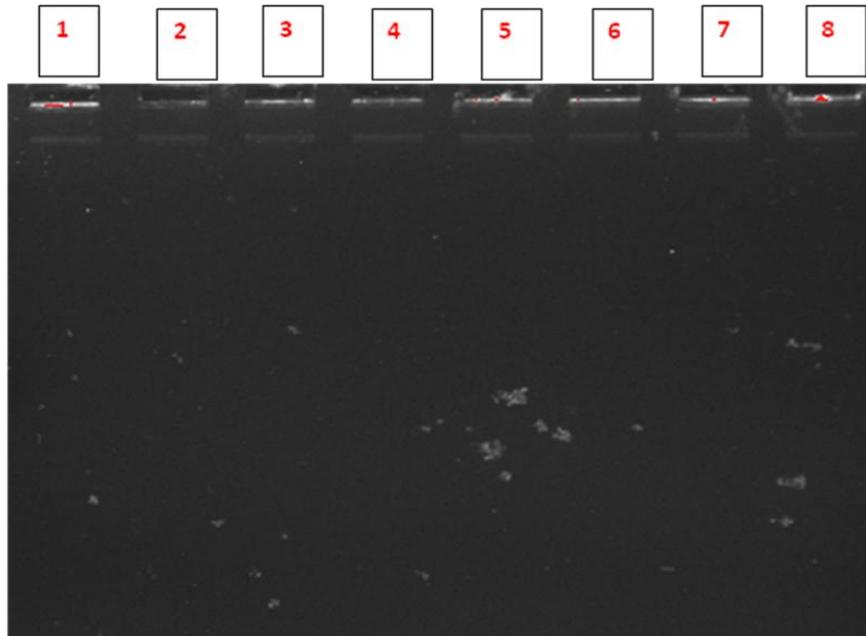


Fig.1, Isolated total DNA with the use of Qaigen kit method in 1% agarose gel photographs.

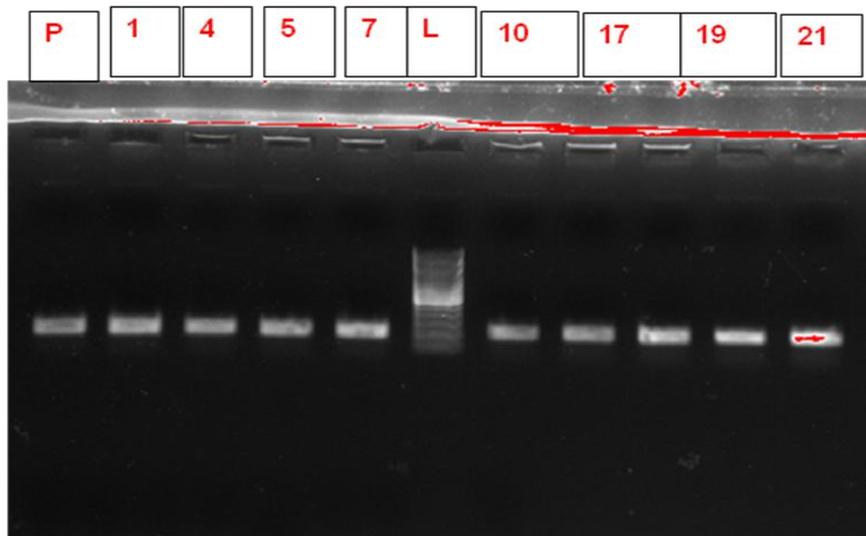


Fig.2, Amplified gene COL9A2 in 1% agarose gel photographs, P indicates for positive control and L correspond to ladder.

Table-1, Biochemical parameter analysis

S.N.	Characteristics	Gender	Cases (N)	Controls (N)	%	χ^2	P value
1.	Gender	Male	50	50	50	2.56	0.001
		Female	50	50	50		
2.	Age	Male	45±2.69	45±2.69	50	1.85	0.11
		Female	47±5.17	47±5.17	50		
3.	BMI	Male	20±0.45	21±3.24	50	1.95	0.05
		Female	21±4.00	18±1.20	50		
4.	Smoking	Male	29±1.57	24±2.82	50	1.56	0.12
		Female	1±4.63	1±2.00	50		
5.	Drinking	Male	22±0.63	24±4.20	50	0.97	0.54
		Female	2±3.01	2±3.24	50		
6.	Manual labour	Male	18±5.12	20±2.46	50	2.65	0.0001
		Female	10±5.97	15±5.20	50		
7.	Family history of inter-vertebral disc disease	Male	30±4.86	2±2.52	50	8.56	0.0001
		Female	25±8.74	4±0.02	50		
8.	Lumbar injury	Male	10±5.31	0±1.65	50	2.85	0.0001
		Female	8±5.98	1±5.20	50		
9.	Schneiderman stage	Male	12±6.52	0±0.00	50	1.26	0.0001
		Female	9±2.58	1±1.23	50		

DISCUSSION

This study investigated the relationship between the COL9A2 Gln326Arg gene detection and development of inter-vertebral 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. [25] The gene COL9A2 encodes a 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. [26-27]

To date, several studies have tested the association between the COL9A2 gene and development of inter-vertebral disc disease in various populations. [28-29] In the literature it has been mentioned that an investigation involving 157 patients and 174 controls, identifying a COL9A2 genetic mutation that co-segregates with the disease phenotype. [30] Similarly in the study it has been reported that a particular polymorphism of COL9A2 is likely to be a less significant susceptibility factor for the development of inter-vertebral disc disease in Greek and Finnish populations. [31] Moreover, in the study has been revealed that COL9A2 gene variation is a cause of this disease in the Indian population. [32] However, it has been found no association between COL9A2 polymorphism and risk of inter-vertebral 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. [33]

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 inter-vertebral disc disease and healthy individuals in the general North Indian 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.

CONCLUSION

The entire study declare about the results suggest that the COL9A2 Gln326Arg gene has involved in the development of inter-vertebral disc disease in the North Indian population, though further research is required to confirm our results.

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Conflicts of interest

There is no any conflict of interest associated with this study.

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