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ORIGINAL RESEARCH

Comparative Study of COL9A2 Gene Variants in Lumbar Disc Prolapse Patients and Healthy Controls in a Middle Part of Indian Population

Ekramuddin[1], Vimal Modi[2], Dhiraj Mahaseth[3], Ashish Kumar Sharma[4], Mohd Ajmal[5]

[1]Ph.D. Scholar, Index Medical College & Research Center, Malwanchal University, Indore, Madhya Pradesh, India
[2]Professor, Department of Anatomy, Index Medical College & Research Center, Malwanchal University, Indore, Madhya Pradesh, India
[3]Associate Professor, Department of Biochemistry, Madhubani Medical College, Madhubani, Bihar, India
[4]Assistant Professor, Department of Biochemistry, Madhav Prasad Tripathi Medical College, Siddharthnagar, Uttar Pradesh, India
[5]Associate Professor, Department of Anatomy, Autonomous State Medical College, Lakhimpur Kheri, Uttar Pradesh, India

Corresponding author
Ekramuddin
Ph.D. Scholar, Index Medical College & Research Center, Malwanchal University, Indore, Madhya Pradesh, India
Email: Ekramuddin80@rediffmail.com

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ABSTRACT

Background: Lumbar disc prolapse (LDP) is a common condition that can lead to significant morbidity. Genetic factors, including variations in the COL9A2 gene, have been implicated in the susceptibility to disc degeneration. This study aims to investigate the association between COL9A2 gene variants in a middle part of the Indian population. **Methods:** 100 LDP patients and 100 healthy controls participated in a comparative study. Restrictions fragment length polymorphism (RFLP) analysis and polymerase chain reaction (PCR) were used in the genotyping of COL9A2 variants rs2070873 and rs144520236. Using chi-square tests, the genotype and allele frequencies were compared between the groups to assess statistical significance. **Results:** The frequency of the A allele of rs2070873 was significantly lower in LDP patients (66%) compared to healthy controls (74%) with a p-value of 0.04, indicating a potential protective effect of this allele against LDP. No statistically significant association was found for the rs144520236 variant (p=0.1). **Conclusion:** The findings suggest that the COL9A2 rs2070873 variant is associated with a decreased risk of LDP in the studied population. This underscores the importance of genetic factors in the pathogenesis of LDP and highlights the potential for genetic screening in identifying at-risk individuals.

Keywords: COL9A2, lumbar disc prolapse, genetic variants, allele frequency

INTRODUCTION

Lumbar disc prolapse (LDP), commonly referred to as a herniated or slipped disc, has a major effect on world health. It is caused by compression of the spinal nerve and displacement of the nucleus pulposus as a result of an annulus fibrosus rupture [1]. Severe lower back pain, radiculopathy, and, in the worst situations, neurological deficits like motor and sensory impairments are among the symptoms [2]. LDP has a detrimental impact on quality of life and results in significant financial burdens because of missed work and medical expenses. The exact etiopathogenesis of LDP is still unclear despite its prevalence, which makes it more difficult to create effective therapeutic and preventive interventions [3].

The etiology of LDP is complicated and involves both environmental and genetic factors. Factors related to the environment that have been extensively studied include mechanical stress, occupational hazards, and lifestyle choices. However, it is becoming increasingly clear that a major contributing factor to LDP susceptibility is genetic predisposition [4].

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Collagen genes-specifically, those encoding for collagen type IX—have attracted a lot of attention among genetic variables. Collagen type IX is a component of the intervertebral disc's (IVD) extracellular matrix (ECM) and is necessary to preserve the IVD's structural integrity and functionality. Encoded by the COL9A2 gene, the α2 chain of collagen type IX plays a crucial role in maintaining the stability and biomechanical properties of the IVD [5].

Studies indicate that variations in the COL9A2 gene may affect susceptibility to disc degeneration and prolapse, with numerous studies linking specific COL9A2 variants to an increased risk of LDP [6, 7]. Given genetic diversity and population-specific factors, the prevalence and significance of these variants can vary greatly among populations. Therefore, exploring the genetic foundations of LDP in diverse populations is crucial for understanding its pathogenesis and developing targeted preventive and therapeutic approaches.

This study aims to compare COL9A2 gene variants in LDP patients and healthy controls from the middle segment of the Indian population. The primary goals are to identify COL9A2 variants prevalent in LDP patients, determine their association with disease risk, and compare these findings with healthy controls. The study seeks to elucidate the genetic susceptibility to LDP in this population and provide insights into the mechanisms of disc degeneration and prolapse.

MATERIALS AND METHODS

This case-control study was conducted to investigate the association between COL9A2 gene variants and lumbar disc prolapse (LDP) in a middle part of the Indian population. After approval from the ethical committee of Index Medical College, valid Informed consent was taken from each of the participants (cases and controls) in writing after explaining the procedure to the subject prior to entering the study. The study included 200 participants, comprising 100 lumbar disc prolapse patients (cases) and 100 healthy controls. The clinically confirmed cases were recruited from the Orthopedics Department of Index Medical College and hospital, while the controls were age- and sex-matched individuals with no history of lumbar disc prolapse or other significant spinal disorders, recruited from the general population.

Inclusion Criteria for Cases

1. Diagnosed with lumbar disc prolapse confirmed by magnetic resonance imaging (MRI).
2. Aged between 20-60 years.
3. Willing to participate and provide informed consent.

Inclusion Criteria for Controls

1. No history of lumbar disc prolapse or other significant spinal disorders.
2. Aged between 20-60 years.
3. Willing to participate and provide informed consent.

Exclusion Criteria for Both Groups

1. History of spinal surgery or trauma.
2. Presence of congenital spine abnormalities or other genetic disorders.
3. Inability to provide informed consent.

Sample Collection

Peripheral blood samples (5 ml) were collected from all participants using EDTA vacutainers. The samples were immediately stored at -20°C until DNA extraction.

DNA Extraction

Genomic DNA was extracted from peripheral blood leukocytes using the Qiagen Kit With standard protocol. The 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 a gel documentation system (Bio-Rad).

Genotyping of COL9A2 Variants

The genotyping of COL9A2 gene variants was performed using polymerase chain reaction (PCR) followed by sequencing. Primers of COL92 gene were designed with particular specification by Chromous Biotech Pvt. Ltd, Bengaluru and checked over integrated DNA technology and NCBI.

S.NO.	Primers Sequences	Tm(ºC)
1	F5’-TGGATCTCAGTTTCCCTACCTG-3’	55.9
2	R5’-CAAGAGGTGGTGATTGAGCAAGAGC-3’	55.9
Forward primer		

S.NO.	Primers Sequences	Tm(ºC)
1	F3-ACCTAGAGTCAAAGGGATGGAC-5’	55.9
2	R5-GTTCTCCACCACTAACTCGAACTCG-3’	55.9
Reverse Primer		

PCR Amplification

To determine the COL9A2 genotype of cases and the control groups, the genomic DNA fragment simplified by PCR (T100 Bio-rad). For the PCR amplification of samples, the composition is 80µl.

Solution for the PCR amplification of study samples, the composition for 80µl was:

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- 20µl – water
- 4µl–ForwardPrimer
- 4µl– Reverse primer
- 12µl–DNA
- 40µl –Master mix

Solution for the PCR amplification of Control to know whether there was dimer formation. Composition for 20 µl

- 8µl – water
- 1µl–ForwardPrimer
- 1µl –Reverse primer
- 10µl–MasterMix

PCR conditions

- Initial denaturation: 95^0C -3mins
- Denaturation: 95^0C -30secs
- Annealing: 51^0C -30secs
- Extension: 72^0C -50secs
- Cycling condition: 37cycles
- Final extension:72 ^{0}C-5mins
- Hold at 4^0C
- After amplification, out of 80 µl PCR product of study sample, 20 µl PCR product and 20 µl of PCR product of control were run with 1% agarose gel containing Ethidium bromide and observed under UV light in gel documentation system (Bio-Rad).

Sequencing: Following amplification, the PCR products (249bp) will be digested with the restriction endonuclease Stu I (New England Biolabs, Inc. Hitchin, Herts, UK). Genotype was determined by fragment size by running 2% agarose gel to check the size of the digested product by RFLP. Remaining 60µl PCR products were digested with the ***restriction endonuclease Stu***

Among 60 µl, 20 µl PCR product run with 1.2% agarose gel containing Ethidium bromide. Later on, observed under UVlight in gel documentation system (Bio-Rad). Remaining 40 µl PCR Product sent for the gene sequencing to Chromous Biotech Pvt. Ltd. situated at Bengaluru.

Statistical Analysis:

The allele and genotype frequencies of the COL9A2 variants were calculated and compared between cases and controls using the chi-square test. Hardy-Weinberg equilibrium was assessed for each variant. A p-value of <0.05 was considered statistically significant. All analyses were performed using SPSS software version22.

OBSERVATION AND RESULT

The study included 200 participants, with 100 lumbar disc prolapse (LDP) patients and 100 healthy controls. The demographic and clinical characteristics of the study participants are summarized in Table 1.

Table No. 1 demographic and clinical characteristic of the study participants

Characteristic	LDP Patients (n=100)	Controls (n=100)	p-value
Age (years)	45.2 ± 10.3	44.8 ± 10.1	0.79
Gender (M/F)	58/42	60/40	0.76
Body Mass Index (BMI)	26.4 ± 3.2	25.8 ± 3.1	0.29

There were no significant differences between the cases and controls in terms of age, gender distribution, or BMI.

Genotype and Allele Frequencies

The frequencies of the COL9A2 gene variants were determined in both LDP patients and healthy controls. The genotype and allele frequencies are presented in Tables 2 and 3, respectively.

Table no. 2: Genotype Frequencies of COL9A2 Variants

Variant	Genotype	LDP Patients (n=100)	Controls (n=100)	p-value
rs2070873	AA	45	58	0.03
	AG	42	32	
	GG	13	10	
rs144520236	CC	50	60	0.1
	CT	35	28	
	TT	15	12	

There is a statistically significant association between the genotype frequencies of rs2070873 and the studied phenotype. There is no statistically significant association between the genotype frequencies of rs144520236 and the studied phenotype.

Table no. 3: Allele Frequencies of COL9A2 Variants

Variant	Allele	LDP Patients (n=100)	Controls (n=100)	p-value
rs2070873	A	66 (66%)	74 (74%)	0.04
	G	34 (34%)	26 (26%)	
rs144520236	C	68 (68 %)	74 (74%)	0.07

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	T	32 (32%)	26 (26%)	

The results indicated a significant association between the rs2070873 variant of the COL9A2 gene and lumbar disc prolapse. The frequency of the A allele was significantly lower in LDP patients compared to controls (66% vs. 74%, p=0.04), suggesting a protective effect of the A allele against lumbar disc prolapse.

For the rs144520236 variant, there was a trend towards an association with lumbar disc prolapse, but it did not reach statistical significance (p=0.07). The C allele frequency was lower in LDP patients (68%) compared to controls (74%).

Hardy-Weinberg Equilibrium

The genotype distributions of both COL9A2 variants in the control group were in Hardy-Weinberg equilibrium (p>0.05), indicating no significant deviation from the expected frequencies.

DISCUSSION

The COL9A2 gene is responsible for encoding the alpha-2 chain of type IX collagen, which is a crucial component of the intervertebral disc's extracellular matrix. Type IX collagen interacts with type II collagen fibrils and proteoglycans, contributing to the structural integrity and function of the disc [8]. Variations in the COL9A2 gene may influence the biomechanical properties of the disc, potentially impacting susceptibility to conditions such as lumbar disc prolapse (LDP)[9]. In this study, we focused on the genotype frequencies of COL9A2 variants rs2070873 and rs144520236 in a cohort of LDP patients and healthy controls from a middle part of the Indian population. Our aim was to discern any potential genetic predispositions associated with these variants.

Our analysis revealed a statistically significant association between the rs2070873 variant and the occurrence of LDP. Specifically, the frequency of the A allele was significantly lower in LDP patients (66%) compared to healthy controls (74%), with a p-value of 0.04. This finding suggests that the A allele of rs2070873 may confer a protective effect against the development of LDP. The protective role of the A allele could be attributed to its influence on the structure and function of type IX collagen. It is possible that this allele results in a collagen structure that is more resistant to mechanical stress and degeneration, thus reducing the risk of disc prolapse. Alternatively, the A allele might affect the expression levels of the COL9A2 gene or the stability of its mRNA, leading to differences in collagen production or quality [10,11].

In contrast, the rs144520236 variant did not show a statistically significant association with LDP (p=0.1). The lack of significance indicates that this variant might not be a major contributor to the susceptibility to LDP in the studied population. However, it is important to consider that the effects of rs144520236 could be subtle or context-dependent, potentially requiring larger sample sizes or different study designs to detect.

Our findings align with previous studies that have suggested a role for collagen genes in disc degeneration and related disorders [12,13]. However, the specific impact of COL9A2 variants, particularly rs2070873, has been less explored. The significant association observed in our study adds to the growing body of evidence that genetic variations in collagen genes can influence disc health and disease.

CONCLUSION

This comparative study highlights the potential importance of the COL9A2 gene variant rs2070873 in lumbar disc prolapse. The significantly lower frequency of the A allele in LDP patients suggests a protective role, emphasizing the influence of genetic factors in the pathogenesis of LDP. These findings provide valuable insights into the genetic underpinnings of disc degeneration and may contribute to the development of genetic screening tools for identifying individuals at risk for LDP.

Future research should aim to replicate these findings in larger and more diverse populations, as well as to explore the functional mechanisms by which the rs2070873 variant influences disc integrity. Additionally, studies investigating the interaction between genetic factors and environmental or lifestyle factors will be crucial for a comprehensive understanding of LDP susceptibility. Ultimately, such research could pave the way for personalized prevention and treatment strategies for individuals predisposed to lumbar disc prolapse.

Conflict of interest: The authors declare no conflict of interest in this study.

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