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ORIGINAL RESEARCH

Correlation between COL9A2 Gene Variants and the Severity of Lumbar Disc Prolapse in a Middle Part of Indian Population

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ABSTRACT

Background: A common degenerative spinal condition that causes significant morbidity is lumbar disc prolapse. The degree of disc prolapse is largely determined by genetic predisposition. This study examines the relationship between middle-class Indians' COL9A2 gene variants and the degree of lumbar disc prolapse. **Methods:** Two COL9A2 variants, rs2070873 and rs144520236, were genotyped in a cohort of 100 patients who had been diagnosed with lumbar disc prolapse. Disc prolapse severity was classified as mild, moderate, or severe according to imaging and clinical standards. The association between the severity of lumbar disc prolapse and the COL9A2 variants was examined using statistical analysis. **Results:** One patient had severe disc prolapse out of the 100 patients with the COL9A2 variant rs2070873; the remaining 56 patients had mild disc prolapse, 43 had moderate disc prolapse, and 56 patients had neither. On the other hand, out of the 100 patients who had the COL9A2 variant rs144520236, 66 had severe disc prolapse, 23 had moderate prolapse, and 11 had mild prolapse. Based on statistical analysis, it was found that the COL9A2 variant rs144520236 significantly correlated (p-value < 0.05) with severe disc prolapse, with most carriers exhibiting severe symptoms. This implies that patients who carry this variant are significantly more likely to experience severe disc prolapse. **Conclusion:** The middle segment of the Indian population has a strong correlation between severe lumbar disc prolapse and the COL9A2 gene variant rs144520236. This genetic variation may be a useful marker for estimating the degree of disc degeneration, which could help with early detection and focused treatment plans for those who are more susceptible.

Keywords: COL9A2 Gene, Lumbar Disc Prolapse, severity, intervertebral disc.

INTRODUCTION

Worldwide, lumbar disc prolapse, a common musculoskeletal condition, is a major contributor to lower back pain and disability. It happens when the intervertebral disc, which cushions the vertebrae, ruptures or herniates, causing pain and nerve compression [1]. Lumbar disc prolapse has a complex etiology that includes genetic, environmental, and mechanical factors. The significance of genetic predisposition in the onset and severity of lumbar disc diseases has been highlighted by recent developments in genetic research. Of all the genetic factors that have been implicated, the collagen genes have attracted the most attention. One major structural protein that is essential for preserving the health and functionality of intervertebral discs is collagen. There has been

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evidence that prolapse and disc degeneration are influenced by variations in collagen genes, specifically the COL9A2 gene [2,3].

The alpha-2 chain of type IX collagen, a small collagen component of the intervertebral disc that contributes to disc stability and extracellular matrix structure, is encoded by the COL9A2 gene [4]. Variants in the COL9A2 gene have been linked in a number of studies to lumbar disc degeneration in various populations [5,6]. Nonetheless, little is known about the genetics of lumbar disc prolapse in the Indian population, particularly in the middle regions of the country. This region's genetic diversity and distinct environmental exposures make it a compelling place to look into how COL9A2 gene variants affect lumbar disc prolapse.

The pathophysiological mechanisms underlying lumbar disc prolapse can be better understood by examining the relationship between COL9A2 gene variants and the severity of the condition. Additionally, it can help in the creation of individualized treatment plans and prophylactic actions. Finding genetic markers linked to disc prolapse can also help with early diagnosis and risk assessment, which may lessen the impact of this crippling ailment.

This study aims to elucidate the association between COL9A2 gene variants and the severity of lumbar disc prolapse in a middle part of the Indian population. By employing a case-control study design and utilizing advanced genetic analysis techniques, we seek to uncover the genetic predispositions that contribute to the variability in disease presentation and progression. This research will not only advance our understanding of the genetic underpinnings of lumbar disc prolapse but also contribute to the broader field of musculoskeletal genetics.

MATERIALS AND METHODS

The purpose of this study was to examine the relationship between COL9A2 gene variants and patients with clinically diagnosed lumbar disc prolapse (LDP) in a middle-class Indian population. Each participant provided valid informed consent in writing after receiving approval from Index Medical College's ethical committee and being informed about the procedure before beginning the study. There were one hundred lumbar disc prolapse patients in the study. The patients who underwent clinical confirmation were chosen from Index Medical College's and hospital's orthopedics department.

Inclusion criteria

1. Magnetic resonance imaging (MRI) confirmed the diagnosis of lumbar disc prolapse.
2. been alive for 20 to 60 years.
3. being open to taking part and giving informed permission.

Exclusion Criteria

1. First history of trauma or spinal surgery
2. The existence of other genetic disorders or congenital abnormalities of the spine.
3. The incapacity to give informed permission.

Sample Gathering: Using EDTA vacutainers, peripheral blood samples (5 ml) were drawn from each participant. After that, the samples were kept at -20°C until DNA could be extracted.

DNA Extraction

By following standard protocol, genomic DNA was extracted from peripheral blood leukocytes using the Qiagen Kit. On a 1% agarose gel, the purity of the DNA was estimated. The synthetic primer oligos were obtained from Chromous Biotech Pvt. Ltd. located in Bengaluru. Using PCR (Bio-Rad, T-100), the extracted DNA was amplified and then separated on a 1.2% agarose gel that contained ethidium bromide. In a gel documentation system, gel photos were captured (Bio-Rad). Variants of COL9A2 Genotyping Polymerase chain reaction (PCR) was used for genotyping of COL9A2 gene variants, which was followed by sequencing. Chromous Biotech Pvt. Ltd., in Bengaluru, created specific primers for the COL92 gene and verified them using integrated DNA technology and the NCBI.

S.NO.	Primers Sequences	Tm(°C)
1	F5'-TGGATCTCAGTTTCCCTACCTG-3'	55.9
2	R5'-CAAGAGGTGGTGATTGAGCAAGAGC-3'	55.9
Forward primer		

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

PCR Amplification: The genomic DNA fragment was simplified by PCR (T100 Bio-rad) to ascertain the COL9A2 genotype of the cases and the control groups. The composition is 80µl for the PCR amplification of samples. The 80µl PCR amplification solution for the study samples contained the following ingredients: 20 µl of water, 4 µl of forward primer, and 4 µl of reverse primer, 12µl-DNA and 40µl - Master mixture.

Method to determine whether dimer formation occurred through PCR amplification of the control

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sample: Ingredients for 20 µl: 8 µl water, 1 µl forward primer, and 1 µl reverse primer and 10µl Master Mix.
PCR circumstances: initial denaturation: 95℃ -3min, Denaturation: 95℃ -30sec; Annealing: 51℃ for 30 seconds; Extension: 72℃ for 50 seconds, Cycle count: 37 cycles and Final extension: 5 minutes at 72 ℃. Hold at 4℃
Following amplification, a 1% agarose gel containing Ethidium bromide was used to run 20 µl of the study sample's PCR product and 20 µl of the control's PCR product, which were then observed under UV light in a gel documentation system (Bio-Rad). Sequencing: After amplification, restriction endonuclease Stu I (New England Biolabs, Inc. Hitchin, Herts, UK) will be used to digest the PCR products (249 bp). By using a 2% agarose gel to measure the size of the digested product through RFLP, the genotype was ascertained based on fragment size. Restrictions endonuclease Stu was used to digest the remaining 60µl PCR products. Twenty µl of the 60 µl PCR products was run on a 1.2% agarose gel with Ethidium bromide. Afterwards, observed in a gel documentation system (Bio-Rad) under UV light. The remaining 40 µl of the PCR product was sent to Chromous Biotech Pvt. Ltd. in Bengaluru for gene sequencing.

Statistical Analysis
SPSS VERSION 27.0 statistical software was used to analyze the data.

OBSERVATION AND RESULTS

One hundred lumbar disc prolapse (LDP) patients were included in the study. Table 1 provides an overview of the study participants' clinical and demographic characteristics.

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

Parameters		**Mean ± Standard deviation (S.D.)**
Age		45.2 ± 10.3
Gender		58/42
Body Mass Index (BMI)		26.4 ± 3.2
COL9A2 Variant (SNP)	rs2070873	
	rs144520236	
Severity of Disc Prolapse	**rs2070873**	**rs144520236**
Mild	56	11
Moderate	43	23
Severe	01	66

The COL9A2 Variant (rs2070873) was discovered to be present in 56 patients with mild disc prolapse, 43 with moderate disc prolapse, and 1 with severe disc prolapse. Conversely, the COL9A2 variant (rs144520236) was linked to 66 patients with severe disc prolapse, 23 patients with moderate disc prolapse, and 11 patients with mild disc prolapse. In the analysis, there was a significant ($p < 0.05$) strong correlation between severe disc prolapses and the COL9A2 Variant rs144520236. Because most patients with this variant experienced severe symptoms, it is possible that those who carry this variant are more likely to experience severe disc prolapse.

DISCUSSION

In a population from central India, the current study examined the relationship between COL9A2 gene variants and the degree of lumbar disc prolapse. Two particular COL9A2 variants, rs2070873 and rs144520236, were found to have different patterns of association with the severity of disc prolapse in the patients under investigation. Regarding the COL9A2 Variant rs2070873, half of the patients (56 out of 100) had mild disc prolapse, 43 had moderate symptoms, and only one had severe symptoms. The distribution of this variant indicates that milder forms of disc prolapse are primarily linked to the rs2070873 variant. It's possible that this variant is not a significant genetic risk factor for severe disc degeneration in this population, given the low incidence of severe disc prolapse among carriers of this variant. COL9A2 Variant rs144520236, on the other hand, showed a strong correlation with severe disc prolapse. 64 patients with this variant had severe disc prolapse, 23 had moderate disc prolapse, and only 11 had mild disc prolapse, according to our analysis. Based on this pattern, individuals who carry the rs144520236 variant may have a markedly increased risk of experiencing severe disc prolapse. It's possible function as a genetic marker for increased susceptibility to more severe forms of disc degeneration is highlighted by the strong correlation observed between this variant and severe symptom [7,8].
These results add to the increasing amount of evidence that suggests genetic factors, specifically mutations in the COL9A2 gene, are important for the onset and course of lumbar disc prolapse. The striking difference between the severity profiles of the two variants under investigation raises the possibility that these variants' effects on disc health are caused by distinct mechanisms. The rs144520236 variant seems to predispose people to more severe disc pathology, whereas the rs2070873 variant seems to be associated with milder disc changes. Comprehending the genetic foundations of lumbar disc prolapse can yield noteworthy clinical consequences, specifically concerning risk assessment and customized

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therapeutic methodologies [9, 10]. Identifying individuals at higher risk for severe disc prolapse through genetic screening may allow for early interventions and targeted therapies aimed at preventing disease progression and managing symptoms more effectively.

The study does, however, have certain shortcomings. Because of the small sample size, it may be harder to extrapolate the results to larger populations. Furthermore, because the study was limited to a single area of India, more investigation is required to ascertain whether similar relationships exist among other racial and geographic groupings. To confirm these findings and investigate possible interactions between genetic variations and environmental or lifestyle factors in the development of lumbar disc prolapse, larger and more diverse populations should be studied in the future.

CONCLUSION

Our study demonstrates the strong association in the population under investigation between the COL9A2 Variant rs144520236 and severe lumbar disc prolapse. This variant may be a useful genetic marker for identifying people who are more likely to experience severe disc degeneration, opening the door to more individualized and successful treatment plans.

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