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***Research paper***

# Association of single nucleotide polymorphism in the loxl1 gene with pseudoexfoliation syndrome in northwestern Rajasthan

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## Abstract

Purpose: To identify, evaluate and establish the relation of LOXL1 gene polymorphism among patients with pseudoexfoliation syndrome in North West Rajasthan.
Methods: 50 patients diagnosed with pseudoexfoliation syndrome and 50 healthy subjects of age >40 years were enrolled in the study. All participants underwent routine ophthalmic examination. 2ml blood samples were taken of each participant which were sent to Multi Disciplinary Research Unit to be checked for specific gene mutation using Polymerase Chain Reaction technique.
Results and conclusion: Study showed that rs10486(G) SNP and rs21652(T) SNP has been present in general population also but is more prevalent in those with pseudoexfoliation syndrome. While, rs38259(G) SNP was found not to be a significant factor in development of pseudoexfoliation syndrome. Understanding the genetic basis of pseudoexfoliation syndrome would further contribute in aiding the early diagnosis and management of this disease.

***Keywords***: *Genetics; LOXL1; PCR; Pseudoexfoliation; Rajasthan.*

## 1. Introduction

Pseudoexfoliation syndrome was first described by Lindberg, a finnish ophthalmologist in the year 1917. It is defined as an ocular manifestation of an age-related systemic disorder of the extracellular matrix characterized by the production and progressive accumulation of a fibrillar extracellular material in many ocular and systemic tissues.[1] It is called 'pseudoexfoliation' to differentiate it from true exfoliation of anterior lens capsule which occurs due to chronic exposure to infrared radiations.[2]
The average worldwide prevalence of pseudoexfoliation syndrome is 10%–20% of the general population over the age of 60 years.[3] In a rural South Indian population older than 40 years, the prevalence of pseudoexfoliation syndrome is 6%.[4] The risk of pseudoexfoliation syndrome converting to pseudoexfoliation glaucoma over a period of 15 years is about 60%.[3] Clinically pseudoexfoliation syndrome is diagnosed by the presence of whitish, fibrillogranular exfoliation material on the anterior lens surface at pupillary border. This consists of three zones – (a) a central disc, (b) an intermediate clear zone and (c) a peripheral granular zone.[1]
On local ocular examination, we can find deposits of pseudoexfoliative material over the corneal endothelium, iris, anterior chamber angle and in the anterior chamber. In iris, the pupillary ruff is lost due to granular pseudoexfoliative material deposits. Pseudoexfoliative material has also been found to be deposited in the skin and visceral organs like heart, lung, liver, kidney, gall bladder, and cerebral meninges by electron microscopy.[5], [6] It is a systemic disorder with important eye manifestations, including development of open and closed angle glaucoma and of cataract with zonular instability.[7] Other ocular manifestations may be poor pupil dilatation, blood aqueous barrier breakdown, corneal endothelial decompensation and sometimes, retinal vein occlusion. An increasing number of associations with specific systemic disorders, primarily related to vasculopathy, have been reported including transient ischemic attacks, hypertension, angina, myocardial infarction, stroke, asymptomatic myocardial dysfunction, Alzheimer disease, and hearing loss.[8]
The pathogenesis is multifactorial, but in some populations almost all patients with pseudoexfoliation syndrome have single nucleotide polymorphisms in the LOXL1 gene on chromosome 15, which encodes for an enzyme that is involved in cross linking of tropoelastin and collagen and is therefore important for the formation and maintenance of elastic fibres and extracellular matrix.[9]
Some studies reported a marked association between several LOXL1 gene polymorphisms, including rs1048661 and rs3825942, and susceptibility to pseudoexfoliation syndrome development.[10], [11] However, rs1048661 SNP had a protective role against XFS in some reports from China, Japan and Korea.[12]
Nongenetic factors including ultraviolet light exposure, dietary factors, infectious agents, trauma, as well as oxidative stress, hypoxia, and inflammation have also been suggested to act as comodulating external factors.[13]
This motivated us to screen the LOXL1 gene polymorphism for two major reasons:

a) To implicate this gene as a cause of pseudoexfoliation syndrome .
b) To assess the association of pseudoexfoliation syndrome with genetic variation of LOXL1 gene. The present study was conducted to determine whether common sequence variation in the LOXL1 gene plays a role in the development of pseudoexfoliation syndrome among patients of the North West Rajasthan, India.

## 2. Materials and methods

It was a hospital based cross sectional study to assess association of LOXL1 gene polymorphism among patients with pseudoexfoliation syndrome attending OPD in Department of Ophthalmology, S.P. Medical College & Associated group of hospitals. 50 patients diagnosed with pseudoexfoliation syndrome in age group of >40 years of either sex giving consent for the study were included in the study. While patients who either were not willing to give informed consent, or had any ocular trauma or had a confirmed diagnosis of pigment dispersion syndrome or capsular delamination (true exfoliation) were excluded from the study. 50 healthy individuals of age group >40 years of either sex presenting to the outpatient department of this hospital were enrolled as controls.
Each patient underwent a complete ophthalmic examination, clinical examination and lab investigation including visual acuity, refraction, IOP measurement, fundus examination by direct ophthalmoscopy and slit lamp examination and perimetry.
After confirmation of diagnosis and obtaining consent from the patients, 2ml of blood was drawn in an EDTA vial and send to Multi Disciplinary Research Unit for analysis.
Genomic DNA was isolated from the blood samples of patients and controls using genomic extraction kits and quantification of DNA was done at 260/280nm on spectrophotometer. This quantified DNA was used for further analysis. All the samples were amplified with each primer by Polymerase Chain Reaction technique and the amplified DNA and primer sequence was checked again by electrophoresis for the confirmation of specified gene mutation.
All the data that was collected was entered into Microsoft Excel and was analysed with help of SPSS version 15 software and tests of significance considering level of significance as $p < 0.05$.

## 3. Observations and results

This cross-sectional observational study was conducted in the department of Ophthalmology, Sardar Patel Medical College, Bikaner, Rajasthan after the approval by institutional review board. A total of 50 cases of pseudoexfoliation syndrome and 50 healthy related controls were taken. All the participants were evaluated for LOXL1 gene polymorphism. Specific primer sequences were used in forward and reverse directions to study LOXL1 gene polymorphism in different genotypes.
The PCR primers for rs1048661, rs3825942 and rs2165241 were taken and their sequence has been given below (Table 1).

**Table 1:** Primer Sequences Used to Amplify Candidate Genes

| S. No | Primer Name/ID | Primer Sequence 5' to 3' | No. Of Bases | Scale (nmol) |
|---|---|---|---|---|
| 1 | ALLELE G OF rs1048661[R141L] | Forward- 5' AAG GCC AGC ATG GAC AAA GCT AGA 3' | 24 | 10 |
| 2 | ALLELE G OF rs1048661[R141L] | Reverse – 3' GTA GTA CAC GAA ACC CTG GTC GTA GGT 5' | 27 | 10 |
| 3 | ALLELE G OF rs3825942[G153D] | Forward- 5' AAG GCC AGC ATG GAC AAA GCT AGA 3' | 24 | 10 |
| 4 | ALLELE G OF rs3825942[G153D] | Reverse – 3' GTA GTA CAC GAA ACC CTG GTC GTA GGT 5' | 27 | 10 |
| 5 | ALLELE T OF rs2165241 | Forward- 5' TTC TTA GAA TGC AAG ACC TCA GC 3' | 23 | 10 |
| 6 | ALLELE T OF rs2165241 | Forward- 5' TTC TTA GAA TGC AAG ACC TCA GC 3' | 20 | 10 |

6 sets of primers were supplied by Bioserve Biotechnology (India) Pvt. Ltd. The concentration of primer was at 25 nm scale and were in desalted form. We used 6 sets of primers (ID 617745, 617746, 617747, 617748, 617749 and 617750) to amplify genetic material using PCR electrophoresis. The mean age in the case as well in the control group was found to be 63.52 years and the male to female ratio in case group was 24:26 while in control group was 26:28 (p value = 0.688).

**Table 2:** Pathogenic LOXL1 Gene Variant Distribution in Cases and Controls

| S. No. | Primer name | Positive in number of CASES (n=50) | CONTROLS (n=50) | Negative in number of CASES (n=50) | CONTROLS (n=50) |
|---|---|---|---|---|---|
| 1 | Allele G of rs10486 | 47(94%) P value = 0.001 | 9(18%) | 3(6%) | 41(82%) |
| 2 | Allele G of rs38259 | 5(10%) P value = 0.068 | 13(26%) | 45(90%) | 37(75%) |
| 3 | Allele T of rs21652 | 32(64%) P value = 0.001 | 11(22%) | 18(36%) | 39(78%) |

We observed three polymorphisms of LOXL1 gene in study population at allele G of rs10486, allele G of rs38259 and allele T of rs21652 (Table 2). Polymorphism of allele G of rs10486 was most commonly associated with pseudoexfoliation syndrome group in 47(94%) cases polymorphism of allele G of rs38259 was least associated with PXF syndrome in 5(10%) cases. Remaining 32(64%) cases had polymorphism of allele T of rs21652. In 50 healthy controls, 09(18%) subjects were positive for rs10486 gene polymorphism, 13(26%) subjects were positive for rs38259 gene polymorphism and 11(22%) subjects were positive for rs21652 gene polymorphism. The positivity difference in cases and controls of LOXL1 gene polymorphism at allele G of rs10486 (Primer 1, Fig.1) and at allele T of rs21652 (Primer 3, Fig. 2) were statistically significant with a p value of 0.001. However, LOXL1 gene polymorphism at allele G of rs38259 (Primer 2, Fig.3) were not statistically significant as the p value came out to be 0.068.

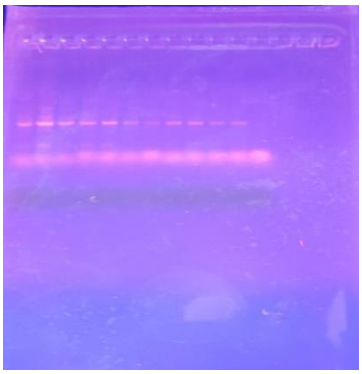

**Fig. 1:** Primer 1 Electrophoresis.

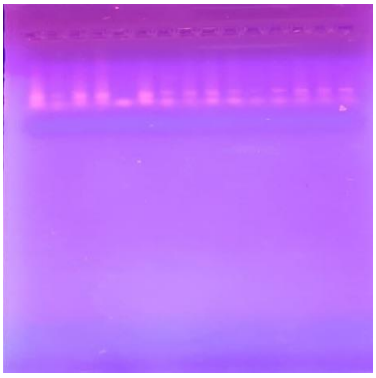

**Fig. 2:** Primer 3 Electrophoresis.

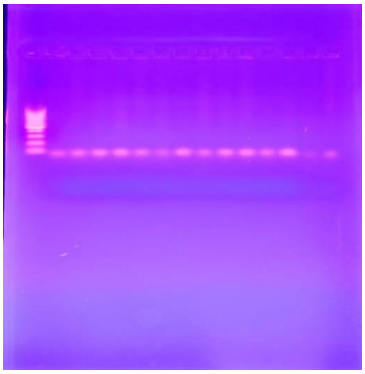

**Fig. 3:** Primer 2 Electrophoresis.

## 4. Discussion

Understanding the genetic basis of pseudoexfoliation syndrome would further contribute in aiding the early diagnosis and management of this disease. Through this study, we had attempted to understand the role of genetic variants of the LOXL1 (Lysyl Oxidase Like 1) gene in order to validate its role in the pathogenesis of pseudoexfoliation syndrome in the population of North West Rajasthan.

The LOXL1 gene family comprises of five genes namely LOXL, LOXL1, LOXL2, LOXL3 and LOXL4.[14] All of the enzymes coded by LOXL1 gene catalyze the extracellular oxidative deamination of lysine residues in elastin, lysine and hydroxylysine in collagen precursor proteins. As a result, certain reactive aldehydes are formed which further form a variety of lysine-derived crosslinks that are required for normal connective tissue functions.[15] Hence, it is understood that LOXL1 is required for maturation of elastin and LOXL1 SNPs develop multiple abnormalities of elastic tissue namely increased laxity of skin, abdominal aortic aneurysms, emphysema and intestinal diverticula.[16]

We identified three LOXL1 (Lysyl Oxidase Like 1) gene polymorphisms among cases and control groups - rs10486(G), rs38259(G) and rs21652(T) LOXL1 gene polymorphisms. Our study showed that all these LOXL1 gene polymorphisms were present in the population of North Western Rajasthan. Pandav et al (2018) conducted a similar study in North Indian population but they did not find any association between LOXL1 single-nucleotide polymorphisms and pseudoexfoliation.[17] In our study:

- rs10486(G) Single Nucleotide Polymorphism was found in 47(94%) cases and 9(18%) control subjects and this difference was statistically highly significant ($p = 0.001$). A study conducted by Panoutsopoulos et al[18] in 2016 also found a significant association for the G allele of rs10486 with high risk of development of PXF syndrome. While, this is in contrast to a study done at Aravind eye hospital by Ramprasad et al [19] in 2007 where they did not find any significant association of PEX syndrome with rs10486 SNP.
- rs38259(G) Single Nucleotide Polymorphism was found in 5(10%) cases and 13(26%) control subjects and the difference was statistically insignificant. Similarly, a study by Min Sagong et al [20] in 2011 in korean population also did not find any association of rs38259 SNP with increased risk of developing PEX syndrome. This is in contrast to a study done by Dubey et al [21] in 2014 in Aravind Eye hospital where they found a significant association of increased risk of PEX syndrome with rs38259 LOXL1 variant in south indian population.

- rs21652(T) Single Nucleotide Polymorphism was found in 32(64%) cases and 11(22%) control subjects and this difference was statistically highly significant (p = 0.001). A similar study conducted by Yaz et al [22] in 2018 in turkish population and concluded that T allele of rs21652 was found to be the most important risk factor for their cohort.

## 5. Conclusion

This study is first of its kind in North West Rajasthan and no such studies have been conducted in North India to the best of our knowledge. Understanding the genetic basis of pseudoexfoliation syndrome would further contribute in aiding the early diagnosis and management of this disease. Our study showed that rs10486(G) SNP and rs21652(T) SNP has been present in general population also but is more prevalent in those with pseudoexfoliation syndrome. While, rs38259(G) SNP was found to be not a significant factor in development of pseudoexfoliation syndrome in our study. There is conflicting evidence for the association of LOXL1 gene polymorphism with pseudoexfoliation syndrome development, with inter – study variability including participants' ethnicity, study design and statistical analysis methods. Studies with large sample size should be done in order to obtain concrete results. Analysis of gene polymorphism was focused on the specific loxl1 gene but analysis of multiple genes will give more insight into the disease pathogenesis.

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