Prevalence of risk alleles in the lysyl oxidase-like 1 gene in pseudoexfoliation glaucoma patients in India

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Purpose: The purpose of this study was to genotype two previously identified SNPs (rs1048661:R141L, and rs3825942:G153D) in the lysyl oxidase-like 1 (LOXL1) gene and determine their association with pseudoexfoliation glaucoma (XFG) in patients from Pune, India. Methods: All subjects underwent detailed phenotyping, and DNA extraction was performed on blood samples by using standardized techniques. Exon 1 of the LOXL1 gene containing the SNPs (rs3825942:G153D; rs1048661:R141L) were Sanger sequenced, and the results were analyzed using sequence analysis software Seqscape 2.1.1. Results: Data were analyzed from 71 patients with XFG and 81 disease-negative, age-matched controls. There was a strong association between the G allele of rs3825942 and XFG with an odds ratio of 10.2 (CI: 3.92–26.6; P < 0.001). The G allele of rs1048661 also showed an increase in risk relative to the T allele (OR = 1.49; CI: 0.88–2.51; P = 0.13), but this was not significant. Haplotype combination frequencies were estimated for rs1048661 and rs3825942; the GG haplotype was associated with a significant increase in risk (OR = 3.91; CI: 2.27–6.73; P < 0.001). Both the GA and TG haplotypes were associated with decreased XFG risk, although the latter was not significant (GA: OR = 0.08; CI: 0.03–0.21; P < 0.001; TG: OR = 0.67; CI: 0.40–1.13; P = 0.13). Conclusion: The risk G allele in rs3825942 (G153D) is strongly associated with the development of XFG in the Western Indian population. Genetic screening strategies to identify LOXL1 risk alleles in the population can assist in case definition and early diagnosis, targeting precious resources to high-risk patients.

Key words: India, latitude, lysyl oxidase-like 1, pseudoexfoliation glaucoma, pseudo-exfoliation syndrome, SNP

Pseudo-exfoliation syndrome (XFS) is a systemic condition associated with open-angle glaucoma that affects over 60 million people worldwide.[1] Pseudoexfoliation glaucoma (XFG) is the most common secondary cause of open-angle glaucoma worldwide.[2,3] XFS is characterized by the deposition of pathological grayish-white extracellular fibrillar protein components (PEX material) in multiple ocular tissues that are composed of constituents of the basement membrane and elastic fiber components.[4] Deposition of this PEX material in the trabecular meshwork obstructs aqueous outflow, and almost 50% of XFS patients will ultimately develop XFG in their lifetime.[3] The ocular phenotype of XFG is more aggressive than primary open-angle glaucoma and is associated with greater elevations in intraocular pressure (IOP) and poorer treatment response.[5]

The prevalence of XFS varies with age and ethnicity,[6,7] with much higher prevalence reported in certain populations, for example, Scandinavian countries and Ireland.[8,9] The role of genetics in the pathogenesis of XFS was confirmed when a genome-wide association study in the Scandinavian population identified three single nucleotide polymorphisms (SNPs) in the lysyl oxidase-like 1 (LOXL1) gene that strongly associated with risk for developing XFS and XFG.[10] LOXL1 is a member of the lysyl oxidase group of enzymes involved in the cross-linking of collagen fibrils and elastin in the extracellular matrix. In the Nordic population, individuals homozygous for the high-risk LOXL1 haplotype in three SNPs (rs1048661, rs3825942, and rs2165241) had an estimated 700-fold increased risk for developing XFG compared to individuals with the...
low-risk haplotype. Although the two non-synonymous SNPs rs1048661 (G>T; Arginine 141 Leucine; R141L) and rs3825942 (G>A; Glycine 153 Aspartate; G153D) in the first exon of the LOXL1 gene confer a higher than 99% population attributable risk for PXS and PXG in the Nordic population, they are associated with different risks in other populations;\(^{[10,11]}\) for example, the A allele of rs3825942 (G153D) in a South-African population showed a stronger association with an increased risk of XFG than the well-reported opposite G allele.\(^{[10]}\)

Given that the LOXL1 risk alleles are reversed in the South-African\(^{[11,12]}\) and Asian populations,\(^{[13,14]}\) understanding the ethnic distributions of LOXL1 alleles is relevant to understand the genetic epidemiology of XFS/XFG. Furthermore, while the proportion of cases and controls harboring LOXL1 risk alleles is relatively consistent across geographic regions, the prevalence of XFG appears to increase with latitude.\(^{[15]}\) There have only been three studies to date examining the prevalence of SNPs in LOXL1 in individuals with XFS or XFG from the Indian population.\(^{[16–18]}\) Two of these studies were based in the Tamil population of South India.\(^{[16,17]}\) The third study was from northern India, based in Chandigarh, Punjab but was underpowered to detect significant genetic associations.\(^{[18]}\) Therefore, the purpose of this study was to sequence two previously identified SNPs (rs1048661; R141L and rs3825942; G153D) in LOXL1 [Fig. 1] and to determine their association with XFG among patients from a population on the Indian subcontinent of higher latitude (Pune, Maharashtra in the Western part of India) and ethnically different from the Tamil population of southern India.

**Methods**

**Patients**

Patients with XFG were recruited following careful phenotyping (by CS) from clinics in Pune, Maharashtra, in western India. The study was approved by the (BLINDED FOR THE PURPOSES OF PAPER REVIEW) and the Indian Medical Research Council (http://www.icmr.nic.in/). Written, informed consent was obtained from all subjects, and the study was performed according to the tenets of the Declaration of Helsinki. All subjects and controls underwent a comprehensive ocular examination, including visual acuity, slit-lamp examination, Goldmann applanation tonometry, and optic disc examination. All subjects underwent a standardized ophthalmic examination, and XFS was identified when the presence of exfoliation material was noted on the lens capsule, iris, or corneal endothelium. Pseudoexfoliation glaucoma was diagnosed if the patient fulfilled the criteria for XFS and the presence of the following: (1) a presenting intraocular pressure (IOP) of >21 mm Hg in at least one eye by Goldmann applanation tonometry; (2) glaucomatous optic nerve head damage on stereoscopic optic disc examination (notching or thinning of the neuroretinal rim and/or increased cup/disc ratio in relation to the optic disc size); (3) an open anterior chamber angle on gonioscopy; (4) reproducible and characteristic glaucomatous visual field defect with the Humphrey 24-2 full-threshold strategy (Carl Zeiss Meditec, Oberkochen, Germany). Ethnic and age-matched control subjects were also recruited from the study population.

**Polymerase chain reaction amplification and DNA sequencing**

Genomic DNA was extracted from all the subjects by using a Wizard Genomic DNA Purification Kit (Promega, Southampton, U.K.) according to the manufacturer’s instructions. In XFG patients and controls, the region of the LOXL1 gene harboring the SNPs, rs1048661 (R141L) and rs3825942 (G153D), was amplified using the primers, 5-ATTCGGCTTTGGCCAGGT-3’ and 5-GAACTGCTGCGGGTAGGA-3. Bidirectional cycle sequencing was performed using BigDye Terminator

![Figure 1: Showing LOXL1 gene with SNPs L141R and G153D (a) and the at-risk haplotypes (b)](image)
Table 1: Genotype and allele frequencies, odds ratios, 95% confidence intervals, and P values for rs1048661 (R141L) and rs3825942 (G153D) in 71 cases of pseudoexfoliation glaucoma and 81 controls

| SNP     | Genotype | Cases n (%) | Controls n (%) | Odds Ratio (95% CI) | P       |
|---------|----------|-------------|----------------|---------------------|---------|
| rs1048661 | GG       | 43 (60.6)   | 39 (48.1)      | 1.93 (0.52-7.01)    | 0.32    |
|         | GT       | 24 (33.8)   | 35 (43.2)      | 1.61 (0.82-3.16)    | 0.17    |
|         | TT       | 4 (5.6)     | 7 (8.6)        | 1.00 (reference)    |         |
|         | G allele | 110 (77.5)  | 113 (69.7)     | 1.49 (0.88-2.51)    | 0.13    |
|         | T allele | 32 (22.5)   | 49 (30.3)      | 1.00 (reference)    |         |
| rs3825942 | GG       | 66 (93.0)   | 40 (49.4)      | 12.5 (4.56-34.5)    | <0.001  |
| G153D   | GA       | 7 (10.0)    | 38 (46.9)      | 1.00 (reference)    |         |
|         | AA       | 0 (0.0)     | 3 (4.8)        | -                   |         |
|         | G allele | 137 (96.5)  | 118 (72.8)     | 10.2 (3.92-26.6)    | <0.001  |
|         | A allele | 5 (3.5)     | 44 (27.2)      | 1.00 (reference)    |         |

*It was not possible to estimate the odds ratio for the AA genotype for G153D as no cases were detected.

Table 2: Estimated haplotype frequency and analysis of missense variants R141L and G153D

| Haplotype | Case n (%) | Control n (%) | Combined n (%) | Odds ratio (95% CI) | P       |
|-----------|------------|---------------|----------------|---------------------|---------|
| GG        | 105 (74)   | 69 (43)       | 174 (57)       | 3.91 (2.27-6.73)    | <0.001  |
| TG        | 32 (22)    | 49 (30)       | 81 (27)        | 0.67 (0.40-1.13)    | 0.13    |
| GA        | 5 (4)      | 44 (27)       | 49 (16)        | 0.08 (0.03-0.21)    | <0.001  |

95% CI: 95% confidence interval

Discussion

Glucoma is the second leading cause of blindness in the adult population in India, affecting 12 million people and is responsible for 12.8% of the total blindness in India. The prevalence of XFS in India has been reported to be between 3% and 6% in those aged 40 years and above. SNPs in LOXL1 have been associated with the development of XFG across multiple populations, but studies have been limited in the Indian population. The results of our study in XFG patients from Maharashtra in western India indicate a strong association between the G allele of rs3825942 (G153D) and XFG, which was statistically significant (OR = 10.2; P < 0.001). However, an association between rs1048661 (R141L) and XFG in our study population was not significant (OR = 1.49; P = 0.13). This is in contrast with the data from original genome-wide association study (GWAS) in XFG in the Scandinavian population in which the “at-risk” allele produced an odds ratio of 2.46 (P = 2.3 × 10⁻¹²). Meta-analyses of published studies in multiple populations implicated rs3825942 (G153D) as the main disease associated SNP in XFG, and this is represented in our genotype data. Furthermore, single-variant analysis in GWAS of XFS cases and controls from 24 countries showed that of all common variants polymorphic across all collections studied, rs3825942 (G > A) remained the most significantly associated (fixed-effects P = 4.14 × 10⁻⁶) but showed very high heterogeneity across the study groups (random-effects P = 0.0039).

Three previous genetic association studies have investigated LOXL1 SNPs and XFS/XFG risk in India. Two of these were based in the Tamil populations from Chennai and Madurai in southern India and the third was from north India. The study from northern India was underpowered (30 XFG cases and 61 controls) and no significant genetic association was detected for rs3825942 or rs1048661). In 52 individuals with XFS (some...
with or without glaucoma) in Chennai, Tamil Nadu, southern India, the G allele of rs3825942 (G153D) showed a significant association ($P = 0.0001$, OR = 4.17, CI: 1.89–9.18) in keeping with our findings. In 150 XFG cases from Madurai, Tamil Nadu, southern India, there was a strong association of the “at risk” G allele in rs3852942 and XFG (OR = 6.40, $P = 2.47 \times 10^{-13}$). The data for the effect of the risk allele in rs1048661 (R141L) and XFG is conflicting in the Indian population and less strongly associated with XFG. No significant association between rs1048661 and XFG was detected in our data which reflects the findings of the Chennai study ($P = 0.156$ for allele G; OR = 1.49) and is in contrast to the data from Madurai (OR = 2.03, $P = 6.77 \times 10^{-5}$). Based on our study and these published studies, the risk G allele in rs3852942 (G153D) is strongly associated with the development of XFG in the Indian population. A consideration that needs to be accounted for while interpreting the results of this study is that the significance of the smaller effect sizes observed may be as a result of the smaller sample size used in this study in comparison to others.

Most previous studies have identified the G allele in rs3852942 (G153D) as the risk allele in the development of XFG.[29] However, the A allele of rs3825942 (G153D) showed a stronger association with an increased risk of XFG in black individuals from the South-African population in contrast to the well-reported opposite G allele.[31] Similarly, other SNPs in LOXL1 show variations in the direction of effect between different populations. The risk-associated allele for rs16958477 (promoter SNP) varied between the South Indian population[17] and the Caucasian population of the United States.[30] The A allele of rs16958477 was associated with an increased risk in Caucasian individuals, whereas in Indian subjects, this allele showed a protective effect.[30]

The reversal of the LOXL1 risk alleles in ethnically different populations[13,14] suggest that while specific LOXL1 alleles are associated with XFG risk, they are not causative.[31] In addition, a large international GWAS study found no common variant in LOXL1 consistently associated across all cohorts, and no common variant in this gene surpassed genome-wide significance in random-effects analysis.[28] This GWAS also identified five new XFS-associated loci that may be implicated in novel biological pathways for disease pathogenesis.[30] Such stark allele reversals and the results from GWAS imply that the genetic architecture underlying XFG disease biology is complex and worthy of further study.[28]

The biological mechanism associated with the risk of allelic variation in the LOXL1 gene and XFG is poorly understood.[32] The LOXL1 SNPs rs1048661 and rs3825942 alter the coding sequence of LOXL1 resulting in amino acid substitutions: R141L (rs1048661; arginine to leucine) and G153D (rs3825942; glycine to aspartate). Overexpression of these mutant LOXL1 proteins (R141L and G153D) in a fibroblastic cell line demonstrated altered LOXL1 processing.[33] However other studies have suggested that the missense changes are not biologically significant[11,13,32,34] and do not affect enzymatic activity.[10] The dysregulation of LOXL1 in XFG may simply be a contributing factor to development of the disease in addition to other factors: raised transforming growth factor beta-1 (TGFβ1), oxidative stress, UV light,[30] and hypoxia.[31,34] There is some emerging evidence that latitude plays a role in the pathogenesis of XFG, but the mechanism of this effect is unknown.[35] In our study, patients from a more northern latitude than the south India studies who carried the risk allele in rs3825942 (G153D) had an OR of 9.90 for XFG compared to 6.40[34] and 4.17[17] in patients from southern India.

**Conclusion**

The findings of this study contribute to the genetic epidemiology of XFG and the role of SNPs in LOXL1 and disease risk. The study has demonstrated that in the Indian population, the G allele in rs3825942 (G153D) confers a significantly increased risk for the development of XFG. Many glaucoma patients already have advanced disease at the time of diagnosis with irreversible visual loss and this is particularly seen in XFG, even in western populations.[30] In the developing world, a major element of any glaucoma strategy must be “case detection.”[37] Genetic screening strategies to identify LOXL1 risk alleles in the population can assist in case definition and early diagnosis, targeting precious resources to high-risk patients.

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**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Ritch R, Schlötzer-Schrehardt U, Konstas AGP. Why is glaucoma associated with exfoliation syndrome? Prog Retin Eye Res 2003;22:253–75.
2. Damji KF. Progress in understanding pseudoexfoliation syndrome and pseudoexfoliation-associated glaucoma. Can J Ophthalmol 2002;37:657–8.
3. Ritch R. Exfoliation syndrome—the most common identifiable cause of open-angle glaucoma. J Glaucoma 1994;3:176–7.
4. Zenkel M, Schlötzer-Schrehardt U. The composition of exfoliation syndrome, a systemic disorder with ocular manifestations. Hum Genomics 2012;6:22.
5. Ritch R, Schlötzer-Schrehardt U. An investigation into LOXL1 variants in black South African individuals with exfoliation syndrome. Arch Ophthalmol 2011;129:206–10.
6. June 2022
7. Vallabh, et al.: LOXL1 in pseudoexfoliation glaucoma in India
8. Hayashi H, Gotoh N, Ueda Y, Nakanishi H, Yoshimura N. Lysyl Oxidase-like 1 polymorphisms and exfoliation syndrome in the
Japanese population. Am J Ophthalmol 2008;145:582-5.

14. Ozaki M, Lee KYC, Vithana EN, Yong VH, Thalathuthu A, Mizoguchi T, et al. Association of LOXL1 gene polymorphisms with pseudoexfoliation in the Japanese. Invest Ophthalmol Vis Sci 2008;49:3976–80.

15. Pasquale LR, Kang JH, Wiggs JL. Consideration for gene-environment interactions as novel determinants of exfoliation syndrome. Int Ophthalmol Clin 2014;54:29–41.

16. Ramprasad VL, George R, Soumitra N, Sharmila F, Vijaya L. Association of non-synonymous single nucleotide polymorphisms in the LOXL1 gene with pseudoexfoliation syndrome in India. Mol Vis 2008;14:318–22.

17. Dubey SK, Kejmanick JF, Krishnasad SR, Sharmila R, Haripriya A, Sundaresan P. Lysyl oxidase-like 1 gene in the reversal of promoter risk allele in pseudoexfoliation syndrome. JAMA Ophthalmol 2014;132:949–55.

18. Pandav SS, Chakma P, Khera A, Chugh N, Gupta PC, Thatteruthody F, et al. Lack of association between lysyl oxidase-like 1 polymorphism in pseudoexfoliation syndrome and pseudoexfoliation glaucoma in North Indian population. Eur J Ophthalmol 2019;29:431–6.

19. Kim S, Kim Y. Variations in LOXL1 associated with exfoliation glaucoma do not affect amine oxidase activity. Mol Vis 2012;18:265–70.

20. Vijaya L, George R, Arvind H, Baskaran M, Raju P, Ramesh SV, et al. Prevalence and causes of blindness in the rural population of the Chennai Glaucoma Study. Br J Ophthalmol 2006;90:407–10.

21. Kumar S, Malik MA, K S, Sihota R, Kaur J. Genetic variants associated with primary open angle glaucoma in Indian population. Genomics 2017;109:27–35.

22. Ramakrishnan R, Nirmalan PK, Krishnasad R, Thulasiraj RD, Tielsch JM, Katz J, et al. Glaucoma in a rural population of Southern India: The Aravind Comprehensive Eye Survey. Ophthalmology 2003;110:1484–90.

23. Dandona L, Dandona R, Mandal P, Srinivas M, John RK, McCarty CA, et al. Angle-closure glaucoma in an urban population in southern India. The Andhra Pradesh eye disease study. Ophthalmology 2000;107:1710–6.

24. Vijaya L, George R, Paul PG, Baskaran M, Arvind H, Raju P, et al. Prevalence of open-angle glaucoma in a rural south Indian population. Invest Ophthalmol Vis Sci 2005;46:4461–7.

25. Aboobaker IF, Johnson WM, Stamer WD, Hauser MA, Allingham RR. Major review: Exfoliation syndrome; advances in disease genetics, molecular biology, and epidemiology. Exp Eye Res 2017;154:88–103.

26. Chen H, Chen LJ, Zhang M, Gong W, Tam PO, Lam DS, et al. Ethnicity-based subgroup meta-analysis of the association of LOXL1 polymorphisms with glaucoma. Mol Vis 2010;16:167–77.

27. Li X, He J, Sun J. LOXL1 gene polymorphisms are associated with exfoliation syndrome/exfoliation glaucoma risk: An updated meta-analysis. PLoS One 2021;16:e0250772.

28. Aung T, Ozaki M, Lee MC, Schlötzer-Schrehardt U, Thorleifsson G, Mizoguchi T, et al. Genetic association study of exfoliation syndrome identifies a protective rare variant at LOXL1 and five new susceptibility loci. Nat Genet 2017;49:1093–1004.

29. Fouti P, Haidich A-B, Chatzikyriakidou A, Salonikou A, Anastasopoulos E, Pappas T, et al. Ethnicity-based differences in the association of LOXL1 polymorphisms with pseudoexfoliation/pseudoexfoliative glaucoma: A meta-analysis. Ann Hum Genet 2015;79:431–50.

30. Fan BJ, Pasquale LR, Rhee D, Li T, Haines JL, Wiggs J. LOXL1 promoter haplotypes are associated with exfoliation syndrome in a U.S. caucasian population. Invest Ophthalmol Vis Sci 2011;52:2372–8.

31. Wiggs JL, Pasquale LR. Expression and regulation of LOXL1 and elastin-related genes in eyes with exfoliation syndrome. J Glaucoma 2014;23 (8 Suppl 1):S62-3.

32. Schlötzer-Schrehardt U, Khor CC. Pseudoexfoliation syndrome and glaucoma: From genes to disease mechanisms. Curr Opin Ophthalmol 2021;32:118–28.

33. Sharma S, Martin S, Sykes MJ, Dave A, Hewitt AW, Burdon KP, et al. Biological effect of LOXL1 coding variants associated with pseudoexfoliation syndrome. Exp Eye Res 2016;146:212–23.

34. Berner D, Hoja U, Zenkel M, Ross JJ, Uebe S, Paoli D, et al. The protective variant rs7173049 at LOXL1 locus impacts on retinoic acid signaling pathway in pseudoexfoliation syndrome. Hum Mol Genet 2019;28:2531–48.

35. Jiwanl A, Pasquale LR. Exfoliation syndrome and solar exposure: New epidemiological insights into the pathophysiology of the disease. Int Ophthalmol Clin 2015;55:13–22.

36. Zenkel M, Krysta A, Pasutto F, Juennemann A, Kruse FE, Schlötzer-Schrehardt U. Regulation of Lysyl Oxidase-like 1 (LOXL1) and elastin-related genes by pathogenic factors associated with pseudoexfoliation syndrome. Invest Ophthalmol Vis Sci 2011;52:8488–95.

37. Thomas R. Glaucoma in India: Current status and the road ahead. Indian J Ophthalmol 2011;59 Suppl (Suppl 1):53–4.