The \textit{LOXL1} Gene Variations Are Not Associated with Primary Open-Angle and Primary Angle-Closure Glaucomas

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\textbf{PURPOSE.} Glaucoma is a complex disease involving multiple genetic factors. Recently, single nucleotide polymorphisms (SNPs) in the \textit{LOXL1} gene have been implicated in exfoliation syndrome (XFS) and exfoliation glaucoma (XFG) but not in the primary glaucomas. This study was conducted to determine the possible involvement of these SNPs in cases of primary open-angle glaucoma (POAG) and primary angle-closure glaucoma (PACG).

\textbf{METHODS.} The three associated SNPs of \textit{LOXL1} (rs1048661, rs19285942, and rs2165241) were screened in 208 unrelated and clinically well-characterized glaucoma cases comprising patients with POAG ($n = 112$) or PACG ($n = 96$) along with 105 ethnically matched normal control subjects from Indian populations. Subjects with exfoliative material on the lens and radial pigmentation in the periphery of the lens that could be earlier signs of XFS were excluded. These SNPs were screened by resequencing and further confirmed by PCR-based restriction digestions. Haplotypes were generated with the three SNPs in cases and control subjects, and linkage disequilibrium (LD) and haplotype analysis were performed with the Haploview software, which uses the EM (expectation-maximization) algorithm.

\textbf{RESULTS.} The SNPs of \textit{LOXL1} did not exhibit any significant association with POAG or PACG, unlike previous studies from Icelandic, Swedish, U.S., and Australian populations with XFS/XFG. Haplotypes generated with these intragenic SNPs did not indicate any significant risk with POAG or PACG phenotypes. The risk haplotype G-G in XFS/XFG in other populations was present in 46% of the normal control subjects in the present cohort.

\textbf{CONCLUSIONS.} The results from the present study do not indicate the involvement of the \textit{LOXL1} SNPs in POAG and PACG. (\textit{Invest Ophthalmol Vis Sci.} 2008;49:2343–2347) DOI:10.1167/ iovs.07-1557

Glaucoma is a group of clinically and genetically heterogeneous optic neuropathies characterized by a gradual and progressive loss of vision.\textsuperscript{2,3} Gonioscopically, primary glaucomas are classified as primary open-angle glaucoma (POAG; OMIM 137750; Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD), which is more common in the Western world\textsuperscript{4,2} and primary angle closure glaucoma (PACG), which is more common among the Asian populations.\textsuperscript{6,7} Both these conditions are characterized by optic nerve head changes, degeneration of retinal ganglion cells, and visual field loss\textsuperscript{8,9} and may be associated with an elevated intraocular pressure (IOP).\textsuperscript{10}

Exfoliation syndrome (XFS; OMIM 177650) is an age-related systemic condition with clinically detectable accumulation of microfibrillar deposits in the lens and anterior segment.\textsuperscript{9,10} The deposition of such material in the trabecular meshwork leads to secondary glaucoma.\textsuperscript{11,12} The prevalence of XFS increases with age, and it may be associated with other vascular conditions.\textsuperscript{9,13,14} The overall prevalence of XFS in population-based studies has been reported from south Indian populations, which varies from 3.0% to 6.0% among subjects over 40 years of age.\textsuperscript{15–17}

POAG exhibits extensive genetic heterogeneity, and 11 chromosomal loci (GLC1A to GLC1K) have been mapped\textsuperscript{18,19} and three genes: myocilin (MYOC; OMIM 601652),\textsuperscript{20} optineurin (OPTN; OMIM 602432),\textsuperscript{21} and WDR36 (OMIM 609669)\textsuperscript{22} have been characterized. Approximately 15 candidate genes have been identified based on case-control association studies but most of these have not been replicated in other populations.\textsuperscript{8}

Recently, it has been shown by genome-wide association studies that single nucleotide polymorphisms (SNPs) in the \textit{LOXL1} gene (OMIM 153456) at 15q24.1 are involved in XFS and XFG.\textsuperscript{23} An extensive screening using a gene microarray (Hap500 Beadchip; Illumina, San Diego, CA) showed that two nonsynonymous SNPs in exon 1 of \textit{LOXL1} (rs1048661 [R141L] and rs3825942 [G135D]) exhibited a strong association with XFS and XFG in two different populations. The initial study conducted on the Icelandic population was later replicated in a Swedish population. Jointly, the two SNPs in exon 1 accounted for >99% of all cases of XFG. It was also shown that an individual with the homozygous risk haplotype (G-G) had a 700 times greater chance of having XFG than those with the low-risk haplotype (G-A).\textsuperscript{23} The significantly strong association of the coding SNPs (rs1048661 and rs3825942) has been independently replicated in two diverse cohorts of Caucasian XFS patients from Australia\textsuperscript{24} and the Midwestern United States.\textsuperscript{25}

XFS has been identified as the most common cause of open-angle glaucoma.\textsuperscript{11} There is also a biological explanation for the association of XFS with weak zonules that may cause anterior movement of the lens, contributing to angle closure and glaucoma.\textsuperscript{12,26} Because glaucoma is a complex disease attributed to multiple gene variants with various magnitudes of effect,\textsuperscript{27} we wondered whether the \textit{LOXL1} SNPs causing XFS...
and XFG may also be associated with primary glaucomas, which may vary between populations. The \textit{LOXL1} SNP (rs2165241) showed a weak association with POAG in the Icelandic population.\textsuperscript{25} However, to the best of our knowledge this has not been studied in the primary glaucomas in other populations. The present study was undertaken to determine the involvement of these XFS and XFG-associated SNPs of \textit{LOXL1} in a cohort of POAG and PACG patients in an ethnically different (Indian) population.

\textbf{METHODS}

\textbf{Clinical Details of the Subjects}

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board. The cohort comprised unrelated, consecutive patients with POAG (\(n = 112\)) and PACG (\(n = 96\)), and 105 normal control subjects, seen at the L. V. Prasad Eye Institute, Hyderabad, India, between January 2002 and March 2007. The diagnoses of POAG and PACG were independently confirmed by two surgeons based on the following inclusion and exclusion criteria mentioned in our preceding publication.\textsuperscript{28} In addition, we looked for exfoliative material and also for radial pigmentation in the periphery of the lens (that could be an earlier sign of XFS) on a dilated slit lamp examination.

Ocular hypertension, normal-tension glaucoma, lens-induced glaucoma, neovascular and XFG, and secondary-open angle glaucoma were excluded. Other ocular diseases that can lead to secondary glaucoma were also excluded.

Normal adult individuals without any signs or symptoms of glaucoma and other systemic diseases served as control subjects. Their visual acuity ranged from 20/20 to 20/40, and IOP was < 21 mm Hg. Clinical examination on stereo biomicroscopy did not reveal any changes in the optic disc suggestive of glaucoma. This diagnosis was essentially one of exclusion: normal pattern of neuroretinal rim and absence of notching or thinning of the rim, disc hemorrhage, or nerve fiber layer defects. Cup-to-disc ratio suitable for the disc size, asymmetry of cup-to-disc ratio \(\leq 0.2:1\) (corrected for size) and absence of beta zone peripapillary atrophy were ‘soft’ signs. The patients and control subjects were matched in ethnicity and geographical region of habitat.

\textbf{Molecular Analysis}

Peripheral blood samples (5–10 mL) were collected from each subject by venipuncture, with prior informed consent, and DNA was extracted by standard protocols.\textsuperscript{29} The three \textit{LOXL1} SNPs from exon 1 (rs1048661 and rs3825942) and intron 1 (rs2165241) were amplified with these three predesigned primers (Table 1) in a thermal cycler (model 9700; Applied Biosystems, Inc. [ABI], Foster City, CA) at an annealing temperature of 60°C. The amplicons were purified with spin columns (Sigma-Aldrich, St. Louis, MO) and screened by resequencing (BigDye chemistry, ver. 3.1; model 3100 DNA Analyzer; ABI), according to the manufacturer’s protocol. Sequencing analysis software was used to read the individual sequences. Subsets of the patient and control samples were further confirmed by restriction digestion of the amplicons at 37°C overnight with appropriate restriction enzymes (Table 1) according to the manufacturer’s guidelines (New England Biologicals, Beverly, MA). The digested amplicons were electrophoresed on 8% nondenaturing polyacrylamide gels, along with an undigested amplicon that served as an internal control. The band patterns based on the abolition or creation of restriction sites for the three variants (Table 1) were generated, and sizes of the corresponding fragments were visualized with the help of a 100-bp DNA ladder (Fermentas, Hanover, MD). The genotypes were directly scored from the gels and correlated with the sequencing data. Each experiment was repeated independently by two investigators who were masked to the phenotypes.

\textbf{Statistical Analysis}

Haploview software that incorporates the EM (expectation-maximization) algorithm was used to determine the maximum-likelihood estimates of allele frequencies, Hardy-Weinberg equilibrium, and haplotype frequencies from the genotype data at the three SNP loci.\textsuperscript{30} Pair-wise linkage disequilibrium (LD) between the individual SNPs was calculated with the LD plot function of the software. The \(\chi^2\) analysis was used to assess the test of significance between the allele and genotype frequencies. The odds ratios were calculated to assess the risk of the individual alleles and genotypes of the three SNPs.

\textbf{RESULTS}

\textbf{Distribution of the \textit{LOXL1} SNPs in POAG and PACG}

The study cohort conformed to Hardy-Weinberg equilibrium. The distributions of the allele frequencies for the three SNPs and their corresponding odds ratios are provided in Table 2. As is evident from the table, the frequencies of the XFS/XFG-
associated alleles were not significantly different between the POAG and PACG cohorts and control subjects. The genotype frequencies of these alleles also did not exhibit any significant difference across the three LOXL1 SNPs in the POAG and PACG cohorts (Table 3).

**LD and Haplotype Analysis at the LOXL1 Locus**

The three intragenic SNPs were typed at the LOXL1 locus to generate haplotypes among the cases and control subjects. Pair-wise LD analysis indicated a strong LD (i.e., $D^*$) between rs1048661 and rs3825942, and $D^*$ was 0.93 between rs3825942 and rs2165241; data not shown).

Four different haplotypes (with frequency >5%) were generated with these three SNPs among POAG and PACG cases and control subjects. There were no significant differences in the haplotype frequencies between the POAG and PACG cases compared with those in the control subjects (Table 4). The results were consistent even after reanalysis of the haplotype data with the two XFS/XFG-associated LOXL1 SNPs (rs1048661 and rs3825942).

**DISCUSSION**

The exfoliation syndrome is an age-related condition characterized clinically by the progressive deposition of fibrillar material throughout the anterior segment. Glaucoma occurs more commonly in eyes associated with XFS; such patients are also predisposed to PACG.9 The LOXL1, which belongs to the family of lysyl oxidase proteins, performs multiple functions in different tissues and is involved in a variety of disorders. It has been suggested that the formation of the extracellular matrix (ECM) of the eye is based on the expression of LOXL1 in the ocular tissues that may be involved in the ECM formation. It has been speculated that the chronic accumulation of the abnormal fibrillar material in the trabecular meshwork can lead to an increase in IOP that would eventually predispose to glaucoma.9,11,12,26 Morphometric and ultrastructural evidence suggest that the deposition of the exfoliation material in the juxtacanalicular area may lead to the development of glaucoma.41 Several in vitro studies have demonstrated the differential expression of various genes at different stages of development in the anterior segment of the eye.42-44 Although the significant involvement of the LOXL1 SNPs with XFS and XFG knockout mice have shown abnormalities in other tissues, but their role in ocular tissues leading to disease pathogenesis is yet to be determined.

To the best of our knowledge, other than the Icelandic and Swedish study, this is the first report to screen for the LOXL1 SNPs in POAG; we also screened for their involvement in PACG. The data from the present study indicated that the three XFS/XFG-associated SNPs were not involved with POAG or PACG. Whereas there was a very mild association of the intronic SNP (rs2165241) with POAG ($P = 0.04$) in the homogeneous Icelandic population, the association was not observed in the relatively heterogeneous POAG ($P = 0.426$) and PACG ($P = 0.262$) populations from India. Overall, the results obtained in the present study were similar to those observed among the patients with POAG from Iceland and Sweden (Table 2). Neither the LOXL1 genotype (Table 3) nor haplotype (Table 4) frequencies exhibited any significant association to POAG or PACG.

The risk haplotype with the rs1048661 and rs3825942 SNPs (G-G) in XFS in other studies was observed in equal frequencies among POAG, PACG, and control subjects in the present study (Table 4). But unlike previous studies, the proportion of T-G haplotype was higher among POAG and PACG cases than among the control subjects (Table 5). It was shown that relative to the low risk G-A haplotype, the G-G and T-G haplotypes conferred substantial risk in XFS and XFG, but the same was not observed in the present cohort (Table 5). Intriguingly, the haplotype supposed to have the lowest risk (T-A) was not observed in the present cohort, similar to all the previous studies. It was also noted that the risk haplotype G-G had a very high frequency in the normal population (~46%) similar to that observed in the general population elsewhere (Table 5).

In summary, we tried to determine the involvement of the XFS/XFG-associated LOXL1 SNPs, in glaucoma pathogenesis based on possible commonalities in the pathophysiology. Morphometric and ultrastructural evidence suggest that the deposition of the exfoliation material in the juxtacanalicular area may lead to the development of glaucoma. Several in vitro studies have demonstrated the differential expression of various genes at different stages of development in the anterior segment of the eye. Although the significant involvement of the LOXL1 SNPs with XFS and XFG...
POAG and PACG share clinical features in the disc and visual field. LOXL1 SNPs in our cohort supports this notion. Although the association of XFS/LOXL1 and other pseudoxfoliation (PXF) syndromes with increased vascular risk highlights their potential role in the disease pathogenesis, their functions are yet uncharacterized. The population-attributable fraction is yet uncharacterized. The population-attributable risk for the high-risk haplotype in the Nordic (99%) and lowan (88%) cohorts strongly suggest that these variants are exclusive of XS and XF. The lack of association with LOXL1 SNPs in our cohort supports this notion. Although POAG and PACG share clinical features in the disc and visual field with XFG, they are indeed more complex disorders, the pathogenesis of which remain to be elucidated.

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**References**


