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Report

# Genetic association of *LOXL1* gene variants and exfoliation glaucoma in a Utah cohort

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**Abbreviations:** ABI, applied biosystems; CEPH CEU, Utah residents with ancestry from northern and western Europe; DNA, deoxyribonucleic acid; Exo1, exonuclease 1; HWE, hardy-weinberg equilibrium; LD, linkage disequilibrium; LOXL1, lysyl oxidase like protein 1; OR, odds ratio; PAR, population attributable risk; PCR, polymerase chain reaction; SAP, shrimp alkaline phosphatase; SNP, single nucleotide polymorphism; SPSS, statistical package for the social sciences; XFG, exfoliation glaucoma; XFS, exfoliation syndrome

**Key words:** exfoliation glaucoma, exfoliation syndrome, *LOXL1* gene, single nucleotide polymorphism, genetics

**Exfoliation glaucoma (XFG) is the commonest identifiable cause of secondary open-angle glaucoma worldwide, characterized by the deposition of fibrillar proteins in the anterior segment of the eye. We investigated *LOXL1* gene variants previously identified to confer susceptibility to XFG in a Utah Caucasian cohort. After a standard eye examination protocol we genotyped SNPs rs2165241 and rs3825942 in 62 XFG or exfoliation syndrome (XFS) patients and 170 normal controls. Genotype frequency distribution, odds ratios (ORs) and population attributable risks were calculated for the risk alleles. The SNP rs2165241 was significantly associated with XFG and XFS ($p = 4.13 \times 10^{-9}$ for an additive model, $OR_{het}$ = 4.42 (2.30 - 8.50), $OR_{hom}$ = 34.19 (4.48 - 261.00); T allele: 83.1% in cases versus 52.4% in controls). Significant association was also found for rs3825942: ($p = 1.89 \times 10^{-6}$). Our findings confirm genetic association of *LOXL1* with XFG and XFS and implicate a potential role of cross linking of elastin in the pathogenesis of XFG. This information will potentially guide glaucoma monitoring efforts by targeting individuals whose genetic profiles put them at higher risk for XFG.**

## Introduction

Glaucoma is the second-leading cause of blindness, and first-leading cause of irreversible blindness in the world.[1] It is projected that 5.9 million people worldwide will be blind in both eyes by 2020.[2] Rather than a single disease, the glaucomas represent several different diseases that all result in vision loss through a final common pathway: optic nerve damage and retinal ganglion cell death.[3] The glaucomas are divided into two major classifications: open-angle and closed-angle. Most patients in the United States with glaucoma have one of the open-angle glaucomas. The pathophysiology of optic nerve damage in these open-angle glaucomas is not totally understood.

The open-angle glaucomas are further subdivided into primary open-angle glaucoma and secondary open-angle glaucomas.[4] Exfoliation glaucoma (XFG) is the commonest identifiable cause of secondary open-angle glaucoma worldwide, characterized by the deposition of fibrillar proteins in the anterior segment of the eye within the setting of exfoliation syndrome (XFS, Fig. 1).[5] XFS is defined as an age-related systemic disease characterized by an accumulation of fibrillar extracellular material in many ocular and non-ocular tissues such as the skin and visceral organs.[6-8] In XFG, it is thought this fibrillar material finds their way into the trabecular meshwork of the eye and cause obstruction of aqueous outflow at the microscopic level. Obstruction of aqueous outflow then leads to elevated intraocular pressure and the characteristic changes of glaucomatous optic neuropathy. In addition, trabecular cell dysfunction, liberated iris pigment blockage of meshwork, and concomitant chronic open-angle glaucoma have been suggested as other mechanisms of glaucoma in XFS.[8] Thus, XFS is closely associated with increased risk of XFG. In a recent study, XFS was found to have a 15-year risk of conversion to XFG by nearly 60%.[9]

Estimates for XFS prevalence in individuals over age 65 vary from 0% in the Inuit population to 7% in the United States to 21% in Greek, Latvian, Russian, Icelandic, Scandinavian and Saudi Arabian populations, which may be due to different race background.[10-12] Several studies suggest that genetic factors may contribute to its pathogenesis, including twins,[13] family aggregation[14,15] and family transmission.[11,15,16]

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Recently, one SNP rs2165241 in intron1 and two SNPs rs1048661 (R141L) and rs3825942 (G153D) in exon1 of the gene *LOXL1* were found to be associated with the XFG and XFS phenotype in Icelandic and Swedish patients.[17] Individuals carrying the highest risk haplotype have over a 100-fold increased risk of developing XFS compared to individuals with the lowest risk haplotype. To investigate and replicate the association of rs2165241 and rs3825942 with XFG and XFS in a different cohort, we genotyped a Utah cohort with 62 cases and 170 age and ethnicity matched controls.

## Results

The demographic and clinical features of this cohort are listed in Table 1. There was no significant difference of sex and age between XFG case and control group. Both SNPs, rs2165241 and rs3825942, which were shown to be in high LD (D' = 1.0) with each other in the CEPH CEU HapMap data, showed significant association with XFG in the Utah cohort. rs2165241 was significantly associated with XFG and XFS ($p = 4.13 \times 10^{-9}$ for an additive model, $OR_{het}$ = 4.42 (2.30 - 8.50), $OR_{hom}$ = 34.19 (4.48 - 261.00); T allele: 83.1% in cases versus 52.4% in controls) and XFS (p = 0.013). Significant association was also found for rs3825942 with XFG and XFS ($p = 1.89 \times 10^{-6}$ ; T allele: 100% in cases versus 85.00% in controls) and XFS (p = 0.024) (Table 2). SNPs, rs2165241 and rs3825942 were shown to be in high LD (D' = 0.87, r2 = 0.14) in our cohort. Haplotype survey revealed that haplotype TG was significantly associated with XFG ($p = 1.25 \times 10^{-9}$ ).

## Discussion

A recent study showed SNPs in the *LOXL1* gene may be involved in the association of XFG. Specifically, rs2165241, located in the first intron and two nonsynonymous SNPs rs1048661 and rs3825942 located in the first exon have significant association with XFG and XFS in Icelandic and Swedish populations. In addition, the two nonsynonymous exon 1 SNPs account for more than 99% of all XFG cases in these Nordic populations.[17] Here we demonstrate SNPs rs2165241 and rs3825942 confer similar risks to XFG and XFS in a US cohort. Considering the population of Utah where our patients were derived has a high Caucasian European ancestry, it is not surprising these patients have similar findings as Thorleifsson et al.,[18] found in their Nordic populations. Our study serves as an independent replication that *LOXL1* confers genetic susceptibility to XFG and XFS in an entirely separate cohort. Our genetic association data in Utah population as well as the original observation in Nordic population[17] support the notion that *LOXL1* gene variants may predispose patients to XFS, rather than XFG. Understanding how patients progress from XFS to XFG will be important in management of this group of patients.

Elastin polymer fibers are formed by the spontaneous cross-linking of tropoelastin. This cross-linking occurs when lysine residues in tropoelastin are deaminated, a reaction that is catalyzed by *LOXL1*. *LOXL1* is one of the five members of the lysyl oxidase protein family, including the LOX protein and the 4 LOX-like proteins, *LOXL1*, *LOXL2*, *LOXL3* and *LOXL4*. All five of the LOX genes exhibit strong homology in exons 2–6 and it is these exons that encode the C-terminal catalytic domain of the peptide. The five LOX proteins differ in exon 1, which encodes a pro-peptide that is cleaved following attachment of the protein to the elastin scaffolding structure. The two coding SNPs in *LOXL1* identified by Thorleifsson et al., cause amino acid substitutions at positions 141 (Arg > Leu) and 153 (Gly > Asp). Both substitutions occur in the N-terminal pro-peptide and are hypothesized to affect either catalytic activity of the enzyme or its ability to bind substrates.

Because *LOXL1* is involved in the cross-linking properties of elastin, *LOXL1* mutations may result in the early breakdown of extracellular matrix and the accumulation of exfoliation material. It is this exfoliation material that can deposit in the trabecular meshwork limiting aqueous fluid drainage and resulting in secondary open-angle glaucoma.[20]

In addition, *LOXL1* mutations may also cause abnormalities in the connective tissues that form the trabecular meshwork, providing an additional reason for secondary open angle glaucoma. Fibulin-5, has been demonstrated to be important for elastic fiber development.[21] Since elastin is an important component of the trabecular meshwork.[22] and *LOXL1* is thought to bind fibulin-5, it is possible that elastin development and regulation could be compromised.[23]

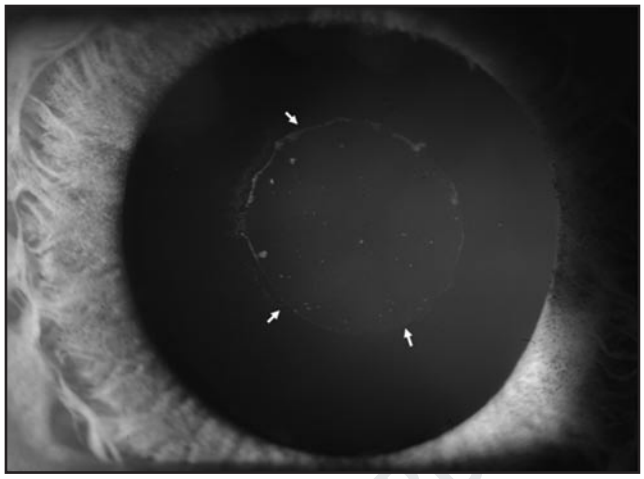

Figure 1. Slit lamp photography of exfoliative glaucoma, demonstrating gray-white fibrillar material deposited on the surface of lens anterior capsule, showed by white arrows. They give the lens an appearance that a layer of the lens capsule is peeled away, or "exfoliating".

Table 1 **Demographics and clinical features of XFG and XFS patients and age and ethnicity matched controls**

| | XFG | XFS | Control |
|---|---|---|---|
| n | 49 | 13 | 170 |
| Gender (m/f) | 20/29 | 4/9 | 90/80 |
| Average age | 75.0 ± 12.9 | 76.7 ± 8.9 | 74.2 ± 8.8 |
| IOP last visit | 14.9 ± 4.8 (on medication) | 16.7 ± 5.1 | 13.4 ± 3.3 |
| Maximum IOP | 28.4 ± 10.4 | 23.9 ± 6.7 | N/A |
| HVF MD | -6.6 ± 7.9 | -0.79 ± 1.7 | N/A |
| CCT | 537.0 ± 34.0 | 546.8 ± 42.4 | 544.4 ± 39.3 |
| C/D | 0.67 ± 0.23 | 0.48 ± 0.19 | 0.18 ± 0.08 |

Abbreviations: IOP, intraocular pressure; HVF, Humphrey visual field; MD, mean deviation; CCT, central cornea thickness; C/D, cup-to-disc ratio.

Table 2 **Association between XFG, XFS and LOXL1 variant rs2165241 and rs3825942 in a Utah cohort**

| SNP | Phenotype (n) | Risk allele | Frq | Trend p | Genotype p | Allele p | ORhom (95% CI) | ORhet (95% CI) | PAR |
|---|---|---|---|---|---|---|---|---|---|
| rs2165241 | XFG (49) | T | 0.85 | $1.86 \times 10^{-8}$ | $2.75 \times 10^{-8}$ | $1.09 \times 10^{-8}$ | 28.49 (3.72–218.49) | 5.39 (2.58–11.22) | 70.68 |
| | XFS (13) | | 0.77 | 0.013 | 0.047 | 0.016 | N/A | N/A | 52.31 |
| | Combination (62) | | 0.83 | $4.13 \times 10^{-9}$ | $1.37 \times 10^{-8}$ | $2.65 \times 10^{-9}$ | 34.19 (4.48–261.00) | 4.42 (2.30–8.50) | 67.83 |
| | Controls (170) | | 0.52 | | | | | | |
| rs3825942 | XFG (49) | G | 1.00 | $1.96 \times 10^{-5}$ | $8.80 \times 10^{-5}$ | $4.53 \times 10^{-5}$ | N/A | N/A | N/A |
| | XFS (13) | | 1.00 | 0.024 | 0.072 | 0.035 | N/A | N/A | N/A |
| | Combination (62) | | 1.00 | $1.89 \times 10^{-6}$ | $8.95 \times 10^{-6}$ | $4.85 \times 10^{-6}$ | N/A | N/A | N/A |
| | Controls (170) | | 0.85 | | | | | | |

ORhom, odds ratio homozygote; ORhet, odds ratio heterozygote; Frq, frequency; PAR, population attributable risk.

With these two possible mechanisms, there is a greater understanding of the process behind how exfoliation syndrome becomes XFG which brings us closer to developing therapeutic interventions.

In addition, it is well known that XFG patients are high risk cataract surgery candidates due to weak zonules,[24] degenerate lens capsules[25] and poor dilation.[26] With the discovery of these SNPs, this will help in identifying those individuals, who are at risk, especially those with very minimal exfoliation syndrome clinical findings that may be overlooked during routine exam but have a strong family history of XFG. This will ultimately aid in planning and avoiding potential surgical complications.

Considering in the US, nearly 12% of all glaucoma patients are due to XFG,[27,28] which can be very difficult to manage, there is a great need to understand the pathophysiology behind XFG to better identify and treat these individuals. This study provides further information that will potentially guide glaucoma monitoring efforts by targeting individuals whose genetic profiles put them at higher risk for XFG.

## Methods

**Patients.** This study was approved by the Institutional Review Boards of University of Utah. All subjects provided informed consent prior to participation in the study. XFG and XFS patients were recruited from the glaucoma clinic at the John Moran Eye Center of the University of Utah Health Science Center. All participants went through a standard examination protocol by a glaucoma specialist. All participants had measurement of visual acuity, pupil examination, slit-lamp examination, determination of intraocular pressure with applanation tonometry and optic nerve and retina examination with pupil dilation. Patients with XFS were identified by the characteristic lens findings exemplified by Figure 1. Patients with XFG had confirmed optic nerve findings consistent with glaucomatous optic neuropathy (including excavation of the optic nerve, thinning of the neuroretinal rim, focal notching, disc hemorrhage) and a corresponding visual field defect on automated perimetry. Normal age-matched controls included individuals with normal slit lamp examinations and normal optic nerves. Clinical and demographic information is listed in Table 1.

**Genotyping.** The Utah cohort of 62 XFG patients was genotyped and allele frequencies were compared to170 ethnicity matched normal controls by lab personnel blinded to case/control status. Two SNPs rs2165241 and rs3825942 were genotyped using an ABI SNPshot method according to the manufacture's manual. A third SNP rs1048661 was not investigated since it was found to be in substantial linkage disequilibrium (D' = 1).[18] In brief description of a SNPshot method, a pair of primers were used for regular PCR, the PCR product was purified by Exo I and SAP (New England Biolabs, Ipswich, MA), then purified PCR product and the SNPshort primer were used to perform SNPshot reaction with SNPshot multiplex mix (Applied Biosystems, Foster City, CA). After one more step of purification using SAP, the product was analyzed on ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA) and the genotyping results were obtained directly from the machine. For the rs2165241 genotype, we PCR-amplified genomic DNA from XFS patients and matched controls using oligonucleotide primers 5'- CCTCTGGGCAGAGAAAACTG-3' and 5'- GAACTAACAGCCCAAAGACAGG-3', with 120 bp PCR product and primer 5'- AGCTCTCAAATGCCACAATA-3' was used as a SNPshort primer. For rs3825942, forward primer 5'- GAACTGCTGCGGGTAGGA-3' and reverse primer 5'- ATTCGGCTTTGGCCAGGT-3' were used for PCR amplification with 188 bp PCR product and primer 5'-CCGTCTCCCAGCAACGGCACGGGG-3" was used as a SNPshort primer. The amplification parameter was 95°C denature 3 minutes first, 94°C 30 seconds, 55°C 30 seconds, 72°C 45 seconds for 35 cycles and 72°C extension additional 10 minutes for both SNPs . All SNPs reported in this manuscript had a genotyping success rate >98% and accuracy >99% as judged by random re-genotyping of 20% of sample in the cohort.

**Analysis for SNPs in *LOXL1* gene.** All SNP genotyping results were screened for deviations from Hardy-Weinberg equilibrium (HWE), and no SNPs showed significant deviation ($p > 0.01$). The chi-squared test for trend for the additive model over alleles was performed to assess evidence for association. Odds Ratios and 95% confidence intervals were also calculated to estimate risk size for the heterozygotes and homozygotes for the risk alleles using logistical regression (SPSS v13.0, SPSS Inc. Chicago, IL). Linkage disequilibrium (LD) and HWE were examined using Haploview v3.32. No significant deviations from HWE were detected. For risk genotypes identified, we calculated population attributable risks (PAR) using the Levin formula.[19]

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