An Investigation Into LOXL1 Variants in Black South African Individuals With Exfoliation Syndrome

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Objective: To investigate the association between 2 lysyl oxidase–like 1 (LOXL1) polymorphisms, rs1048661 (R141L) and rs3825942 (G153D), and exfoliation syndrome (XFS) in black South African individuals.

Methods: A total of 43 black patients with XFS and 47 ethnically matched controls were recruited for genetic analysis. Samples were analyzed for presence of the LOXL1–R141L and G153D variants using restriction fragment length polymorphism analysis. A case-control association study was performed.

Results: The R141L and G153D single-nucleotide polymorphisms (SNPs) were both significantly associated with XFS (P = .00582 and P = .00001, respectively). Consistent with findings in white populations but not in Asian cohorts, the GG genotype of the R141L SNP was present in significantly more XFS cases than controls (P = .00582). However, in this black South African study population, the AA genotype of G153D was present in an overwhelming majority of cases with XFS (P < .00001; odds ratio, 17.10; 95% confidence interval, 4.91-59.56), contrary to all previous articles in which the GG genotype was strongly associated with the disease phenotype.

Conclusion: The LOXL1 SNPs R141L and G153D are significantly associated with XFS in this black South African population. The AA genotype of G153D confers XFS risk in this population, as opposed to the GG genotype described in all other populations, suggesting that unidentified genetic or environmental factors independent of these LOXL1 SNPs may influence phenotypic expression of the syndrome.

Clinical Relevance: Elucidation of the role of genetic factors, including the LOXL1 gene, in XFS will facilitate identification of individuals predisposed to developing this condition.


XFOLIATION SYNDROME (XFS) is a generalized disorder of the extracellular matrix characterized by the pathological deposition and accumulation of fibrillar material throughout the eye. The origin of this fibrillar material is unknown but believed to be derived from abnormal basement membranes of aging epithelial cells in ocular structures. In addition to its occurrence within the eye, exfoliative fibrillopathy has been reported in the skin, blood vessels, and visceral organs, suggesting that XFS may in fact be an ocular manifestation of a systemic disorder.

This condition is associated with an array of ocular manifestations, most frequently a severe and progressive form of chronic open-angle glaucoma. Exfoliation syndrome is acknowledged as the most common identifiable cause of open-angle glaucoma, accounting for approximately 25% of cases worldwide. The prevalence of XFS increases with age, and a number of studies have reported geographical clustering of this condition based on race and ethnicity. Familial aggregation studies have suggested a significant genetic contribution to XFS. Despite these findings, a simple inheritance model is not evident, suggesting that XFS is the result of a complex inheritance pattern with multiple contributing genetic and/or environmental factors.

A landmark genome-wide association study by Thorleifsson et al identified 3 common single-nucleotide polymorphisms (SNPs) in the lysyl oxidase–like 1 (LOXL1) gene on chromosome 15q24.1 that were strongly associated with XFS and exfoliation glaucoma in Scandinavian populations. The LOXL family of proteins play a vital role in the homeostasis...
of elastic tissues, acting as cross-linking enzymes and thereby ensuring spatially defined deposition of elastin fibrils. The identification of the LOXL1 protein in pseu-
dox fibrillation deposits verifies its involvement in abnor-
mal fibrinogenesis in pseuadoxifioative tissues.

Two of these SNPs, rs1048661 (R141L) and rs3825942 (G153D), are located within exon 1 of LOXL1 and cause amino acid missense changes in the protein. This exon codes for the N-terminal portion of the protein, which may have a role in directing the LOXL1 protein to sites of elastogenesis. The third LOXL1 SNP, rs2165241, is located in the first intron of the gene and is presumed not to have any biological consequence. All 3 of these SNPs were in significant linkage disequilibrium in the studied population. These genetic findings have been replicated to a large extent, with some important variations, in numerous studies throughout North America, Australia, Europe, and Asia.

This study investigates the association of these LOXL1 gene polymorphisms with XFS among black South Africans, a geographical cluster with a high prevalence of XFS and exfoliation glaucoma.

METHODS

PATIENT POPULATION

An ethnically matched cohort of 43 elderly black patients with exfoliation syndrome and 47 control individuals were identified from the outpatient ophthalmology service at the East Lon-
don Hospital Complex (Eastern Cape, South Africa) for this study. The study was approved by the Stellenbosch University Committee for Human Research (N08/08/208), and all pa-
tients and controls were recruited after informed consent. All cases and controls underwent an anterior segment evaluation after pupillary dilatation to confirm the presence or absence of the characteristic fibrillar material diagnostic of XFS. Venous blood samples were collected from all study participants.

GENOTYPING

DNA was extracted from the peripheral venous blood samples according to established methods. Polymerase chain reaction primers were designed to amplify the region containing the LOXL1 R141L and G153D variants (forward: 3′-GCA GGT GTA CAG CTT GCT CA-3′ and reverse: 3′-GCG TGG TAG TAC ACG AAA CC-3′), which produced a product of 474 base pairs. Restriction fragment–length polymorphism analysis was used to genotype the 2 SNPs. The Smal (fermentas) and Eco24I (fer-
mentas) restriction endonuclease enzymes were used for R141L and G153D, respectively. Following digestion, the polymer-
ase chain reaction products were resolved on 12% polyacryl-
amide gels and the bands visualized using silver staining. The genotyping method was verified by sequencing randomly se-
lected samples using the BigDye Terminator Sequence Ready Reaction kit version 3.1 (Applied Biosystems, Foster City, California) and analyzed on a 3130xl Genetic Analyzer (Applied Biosystems). BioEdit version 7.0.1 software was used for analysis of the sequencing electropherograms.

STATISTICAL ANALYSIS

A power analysis was performed prior to analysis of the data. It was found that the sample size had sufficient power (85.345%) to detect a clinically significant association between the presence or absence of a specific polymorphism and XFS. Data was analyzed using SAS version 9.1 (SAS Institute Inc, Cary, North Carolina). Descriptive statistics were computed for patient age, and comparison between the mean ages of cases and controls was performed by t test for 2 groups. Furthermore, the pri-
mary outcome variables were analyzed by contingency tables. Associations between the cases and controls and the different allele, genotype, and haplotype frequencies was examined using Pearson χ² test. Odds ratios and relative risk estimates were also produced to examine further interactions between the disease and specific alleles and genotypes. Confidence intervals (CIs) for these estimates were also produced. A P < .05 represented statistical significance in hypothesis testing and 95% confi-
dence intervals were used to describe the estimation of un-
known parameters.

Hardy-Weinberg equilibrium of the allele and genotype frequencies of cases and control subjects was examined, both sepa-
ately and in combination, using χ² and Fisher exact tests.

RESULTS

Of the 43 patients with XFS, 15 had exfoliation glau-
coma. Of the 47 controls, 13 had primary open-angle glau-
coma but no evidence of exfoliation. The cases and con-
trols were age-matched with a mean (SD) age of 72.37 (9.57) in XFS cases and 71.81 (7.56) in controls without XFS, with no significant difference found between the means of these 2 groups (P = .75621).

The GA allele of SNP rs1048661 (R141L) was detected in a statistically higher frequency in patients with XFS than controls (P = .00106). The relative risk of having no disease given the presence of the G allele vs the T allele for this SNP was 0.49 (95% CI, 0.42-0.57). The A allele of SNP rs3825942 (G153D) was strongly associated with exfoliation syndrome (P < .00001) in this sample, with patients being 9.94 times more likely to have an A allele than a G allele (odds ratio, 9.94; 95% CI, 4.75-20.79) (Table 1).

The genotype frequencies for the rs1048661 (R141L) SNP and the rs3825942 (G153D) SNP confirmed statistically significant differences between the XFS cases and controls. The GG genotype of R141L was present in signifi-
cantly more XFS cases than controls (P = .00582), with a relative risk of having no disease (GG vs GT/TT) of 0.46 (95% CI, 0.37-0.59). In this study population, we found that the AA genotype of G153D was present in an overwhelming majority of cases with XFS (P < .00001), with an odds ratio (AA vs GG) of 17.10 (95% CI, 4.91-59.56) (Table 1).

The haplotypes composed of the 2 LOXL1 SNPs rs1048661 and rs3825942 were determined, with the frequencies of the 2-SNP haplotypes differing significantly between the patients with XFS and the controls (P < .00001). The GA haplotype was associated with the highest risk of XFS in which a patient is 9.94 times more likely to have XFS if haplotype GA is present than if either haplotype TG or GG are present (odds ratio, 9.94; 95% CI, 4.75-20.79). The TG haplotype was not detected among the group with XFS (Table 2).

Hardy-Weinberg analysis of allele and genotype frequencies in cases and controls of both SNPs found Hardy-Weinberg equilibrium in all of the subpopulations, with
This study’s participants are representative of the South African Xhosa population, who represent the southernmost extension of the Bantu-speaking nations that began to migrate southwards from an area near present-day Cameroon approximately 4000 years ago. They are fairly homogenous culturally and reside primarily in an area of South Africa known as the Eastern Cape Province. A previous study on genetic substructure in South African Bantu speakers has found that these groups cluster according to linguistic groupings (Xhosa, Zulu, etc.).

Exfoliation syndrome is common in this population; a recent study conducted in a related tribal grouping reported a prevalence of 6.6%. Our finding in the South African Xhosa population is that the AA genotype of the rs3825942 (G153D) SNP is strongly associated with XFS, which is different from the GG genotype noted in all other population groups reported to date. This is unexpected, especially in light of the strength of the associations described previously. In addition, our study association for G153D is with the minor allele, which is distinct from all other articles (Table 3).

A recent publication corroborates the findings of this study. Williams and colleagues investigated $LOXL1$ SNPs in black South African patients with exfoliation glaucoma and also found that the A (and not the G allele) of G153D, in contrast to all other studies, is significantly associated with XFS and exfoliation glaucoma in black South African individuals. An advantage of our compared with that of Williams et al is that our study focused on only 1 ethnic subgroup, the Xhosa-speaking Bantu population (our cases and controls were recruited exclusively from an area where Xhosa-speaking black South African individuals predominantly reside).

The allele frequencies observed in our study compare favorably with the data observed in HapMap (http://hapmap.ncbi.nlm.nih.gov/) and dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) as well as the aforementioned study of another black South African population. For G153D (rs3825942), the G allele has been found at frequencies ranging from 58% to 63% in various black populations in the Southwestern United States, Kenya, Nigeria, and South Africa. The frequency of the G allele observed in the control population in the present study was 62%. This is in contrast with the frequency observed in white populations from the United States and Italy, Asians from China, Japan, and the United States, and Indians from the United States, which ranged from 78% to 88%. For R141L (rs1048661), the frequency that we observed for the G allele of 88% is similar to the other recent study in black South African individuals (81%) but different from that observed in American white (96%) and Japanese individuals (44%). Because R1-41L is not present in HapMap and Williams et al did not evaluate haplotype data, no comparison can currently be made for the haplotype frequencies of the 2 SNPs identified in this study.

Although the allele and genotype frequencies in this study were in Hardy-Weinberg equilibrium for the control groups of both SNPs and the cases in the rs1048661 (R141L) analysis, the allele and genotype frequencies...
for the rs3825942 (G153D) were not in Hardy-Weinberg equilibrium ($P_\text{adj} = 0.0002$) for cases with XFS. In light of the fact that the same genotyping platform was used in all the genetic analyses, and that similar findings have occasionally been described in other case-control studies of XFS involving these same SNPs, it is unlikely that this finding is related in any way to small sample size, inbreeding, or assortative mating of any kind but might be a reflection of an association between this marker and disease susceptibility.

The AA genotype of G153D confers XFS risk in this South African population. This observation, together with findings in several Chinese and Japanese populations that the TT genotype of the rs1048661 (R141L) SNP is strongly associated with XFS contrasts the association of the GG genotype reported in all previously described populations.

In summary, the LOXL1 SNPs rs1048661 (R141L) and rs3826942 (G153D) are significantly associated with XFS in the black South African population. The AA genotype of G153D confers XFS risk in this population, as opposed to the GG genotype reported in all previously described populations.

The fact that the disease-associated haplotype differs across various populations (GA in black South African individuals, TG in Japanese individuals, and GG in white individuals) indicates that they are not the disease-causing variants but that they are in linkage disequilibrium with the actual pathogenic variants.

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Author Contributions: Dr Rautenbach had full access to all the data in the study and takes full responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 3. Comparative Data of LOXL1 Risk Alleles and Minor Allele Frequencies

<table>
<thead>
<tr>
<th>Study (Location)</th>
<th>Allele</th>
<th>Risk Allele</th>
<th>P Value</th>
<th>OR (95% CI)</th>
<th>MAF</th>
<th>Risk Allele</th>
<th>P Value</th>
<th>OR (95% CI)</th>
<th>MAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thorleifsson et al(^a) (Iceland/Sweden)</td>
<td>G</td>
<td>2.3 $\times$ 10^{-2}</td>
<td>2.46 (1.91-3.16)</td>
<td>Iceland 0.349 (T)</td>
<td>Sweden 0.316 (T)</td>
<td>G</td>
<td>3.0 $\times$ 10^{-2}</td>
<td>20.10 (10.80-37.41)</td>
<td>Iceland 0.153 (A)</td>
</tr>
<tr>
<td>Fingert et al(^b) (USA)</td>
<td>G</td>
<td>.00004</td>
<td>NA</td>
<td>0.400 (T)</td>
<td>G</td>
<td>.00303</td>
<td>NA</td>
<td>0.120 (A)</td>
<td></td>
</tr>
<tr>
<td>Fan et al(^c) (USA)</td>
<td>G</td>
<td>.005</td>
<td>1.90 (1.23-2.93)</td>
<td>0.281 (T)</td>
<td>G</td>
<td>1.6 $\times$ 10^{-15}</td>
<td>20.93 (8.06-54.39)</td>
<td>0.205 (A)</td>
<td></td>
</tr>
<tr>
<td>Yang et al(^d) (USA)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>G</td>
<td>4.85 $\times$ 10^{-4}</td>
<td>NA</td>
<td>0.147 (A)</td>
<td></td>
</tr>
<tr>
<td>Aragon-Martin et al(^e) (USA)</td>
<td>G</td>
<td>7.74 $\times$ 10^{-5}</td>
<td>0.442</td>
<td>0.297 (T)</td>
<td>G</td>
<td>3.10 $\times$ 10^{-17}</td>
<td>0.168 (A)</td>
<td>0.202 (A)</td>
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<tr>
<td>Challa et al(^f) (USA)</td>
<td>G</td>
<td>.0222</td>
<td>1.86 (1.10-3.15)</td>
<td>0.335 (T)</td>
<td>G</td>
<td>.00194</td>
<td>3.05 (1.20-7.76)</td>
<td>0.156 (A)</td>
<td></td>
</tr>
<tr>
<td>Hewitt et al(^g) (Australia)</td>
<td>G</td>
<td>8.48 $\times$ 10^{-4}</td>
<td>1.86 (1.27-2.76)</td>
<td>NA</td>
<td>G</td>
<td>7.83 $\times$ 10^{-6}</td>
<td>3.81 (1.54-9.02)</td>
<td>NA</td>
<td></td>
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<tr>
<td>Pasutto et al(^h) (Germany/Italy)</td>
<td>G</td>
<td>2.9 $\times$ 10^{-19}</td>
<td>2.43 (2.00-2.97)</td>
<td>0.348 (T)</td>
<td>G</td>
<td>8.22 $\times$ 10^{-22}</td>
<td>4.87 (3.46-6.85)</td>
<td>0.149 (A)</td>
<td></td>
</tr>
<tr>
<td>Ramprasad et al(^i) (India)</td>
<td>G</td>
<td>.156</td>
<td>1.49 (0.89-2.51)</td>
<td>0.270 (T)</td>
<td>G</td>
<td>.00011</td>
<td>4.17 (1.89-9.18)</td>
<td>0.070 (A)</td>
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<tr>
<td>Lee et al(^j) (China/Singapore)</td>
<td>G</td>
<td>.142</td>
<td>1.38 (0.91-2.08)</td>
<td>0.444 (T)</td>
<td>G</td>
<td>.00180</td>
<td>0.97 (1.48-8.19)</td>
<td>0.082 (A)</td>
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</tr>
<tr>
<td>Chen et al(^k) (China)</td>
<td>T</td>
<td>6.95 $\times$ 10^{-11}</td>
<td>7.59 (3.87-14.89)</td>
<td>0.484 (G)</td>
<td>G</td>
<td>8.00 $\times$ 10^{-4}</td>
<td>NA</td>
<td>0.104 (A)</td>
<td></td>
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<tr>
<td>Fuse et al(^l) (Japan)</td>
<td>T</td>
<td>7.7 $\times$ 10^{-13}</td>
<td>26.0 (18.3-37.1)</td>
<td>0.493 (G)</td>
<td>G</td>
<td>4.1 $\times$ 10^{-4}</td>
<td>NA</td>
<td>0.123 (A)</td>
<td></td>
</tr>
<tr>
<td>Hayashi et al(^m) (Japan)</td>
<td>T</td>
<td>3.0 $\times$ 10^{-10}</td>
<td>99.8 (13.8-722)</td>
<td>0.460 (G)</td>
<td>G</td>
<td>1.4 $\times$ 10^{-5}</td>
<td>NA</td>
<td>0.143 (A)</td>
<td></td>
</tr>
<tr>
<td>Ozaki et al(^n) (Japan)</td>
<td>T</td>
<td>6.41 $\times$ 10^{-4}</td>
<td>17.79 (11.03-28.71)</td>
<td>0.497 (G)</td>
<td>G</td>
<td>1.30 $\times$ 10^{-11}</td>
<td>10.87 (4.39-25.75)</td>
<td>0.137 (A)</td>
<td></td>
</tr>
<tr>
<td>Williams et al(^o) (South Africa)</td>
<td>G</td>
<td>1.7 $\times$ 10^{-4}</td>
<td>23.2 (3.0-177.2)</td>
<td>0.190 (T)</td>
<td>A</td>
<td>5.2 $\times$ 10^{-5}</td>
<td>0.092 (0.045-0.19)</td>
<td>0.380 (A)</td>
<td></td>
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<tr>
<td>Rautenbach et al (South Africa)(^a)</td>
<td>G</td>
<td>.00106</td>
<td>NA</td>
<td>0.117 (T)</td>
<td>G</td>
<td>&lt;.00001</td>
<td>9.94 (4.75-20.79)</td>
<td>0.383 (A)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; MAF, minor allele frequency; NA, data not available; OR, odds ratio.

\(^a\)Present study.
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REFERENCES


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