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.


EXFOLIATION 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 the extracellular matrix by promoting collagen cross-linking and maintaining extracellular matrix integrity.
of elastic tissues, acting as cross-linking enzymes and thereby ensuring spatially defined deposition of elastin fibrils.9,10 The identification of the LOXL1 protein in pseu-
dox fibrinogenesis deposits verifies its involvement in abnor-
mal fibrinogenesis in pseuadoxifibrotic tissues.11,12

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.8 These genetic findings have been replicated
to a large extent, with some important variations,
in numerous studies throughout North America,13,17 Aus-
tria,18 Europe,19 and Asia.20-26

This study investigates the association of these LOXL1
gene polymorphisms with XFS among black South Af-
ricans, a geographical cluster with a high prevalence of
XFS and exfoliation glaucoma.27,28

PATIENT POPULATION

An ethnically matched cohort of 43 elderly black patients with
exfoliation syndrome and 47 control individuals were identi-
fied 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: 5'-GCA GGT GTA
CAG CTT CCT CA-3' and reverse: 3'-GGC CGG 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
digestion. The polymer-
ase chain reaction products were resolved on 12% polyacryl-
imate gels and the bands visualized using silver staining. The
polymerase chain reaction products were resolved on 12% polyacryl-
imate gels and the bands visualized using silver staining. The

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 fre-
cencies 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 G 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 statisti-
cally significant differences between the XFS cases and
controls. The GG genotype of R141L was present in sig-
nificantly 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 consisted of the 2 LOXL1 SNPs
rs1048661 and rs3825942 were determined, with the fre-
cuencies 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
haplotypes 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 fre-
cuencies in cases and controls of both SNPs found Hardy-
Weinberg equilibrium in all of the subpopulations, with
the exception of the G153D cases (P = .00002). This was confirmed using both \( \chi^2 \) and Fisher exact tests and after checking for genotyping errors.

**COMMENT**

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 G allele 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, in our study the association for G153D is with the minor allele, which is distinct from all other articles.

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.

Table 1. **LOXL1** Allele and Genotype Frequencies by Single-Nucleotide Polymorphism in XFS Cases and Controls

<table>
<thead>
<tr>
<th></th>
<th>XFS</th>
<th>Controls</th>
<th>( P ) Value (( \chi^2 ))</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R141L (rs1048661)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>86 (100)</td>
<td>83 (88.3)</td>
<td>.0106</td>
<td>0.49 (0.42-0.57)</td>
</tr>
<tr>
<td>T</td>
<td>0 (0)</td>
<td>11 (11.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>43 (100)</td>
<td>37 (78.7)</td>
<td>.0582</td>
<td>0.46 (0.37-0.59)</td>
</tr>
<tr>
<td>GT</td>
<td>0 (0)</td>
<td>9 (19.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>0 (0)</td>
<td>1 (2.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>G153D (rs3825942)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>74 (86.0)</td>
<td>36 (38.3)</td>
<td>.0001</td>
<td>9.94 (4.75-20.79)</td>
</tr>
<tr>
<td>G</td>
<td>12 (14)</td>
<td>58 (61.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>36 (83.7)</td>
<td>8 (17.0)</td>
<td>.0001</td>
<td>17.10 (4.91-59.56)</td>
</tr>
<tr>
<td>GA</td>
<td>2 (4.7)</td>
<td>20 (42.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>5 (11.6)</td>
<td>19 (40.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio; RR, relative risk; XFS, exfoliation syndrome.

Table 2. **LOXL1** Haplotypes in XFS Cases and Controls

<table>
<thead>
<tr>
<th>rs1048661-rs3825942 Haplotype</th>
<th>XFS</th>
<th>Controls</th>
<th>( P ) Value (( \chi^2 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>74</td>
<td>36</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>GG</td>
<td>12</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>0</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: XFS, exfoliation syndrome.
for the rs3825942 (G153D) were not in Hardy-Weinberg equilibrium ($P = .00002$) 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, as well as in similar studies of other conditions, it is our opinion that this is a real finding despite deviation from Hardy-Weinberg equilibrium. We are, however, uncertain of its implications regarding Hardy-Weinberg assumptions in our population. As the sample population is identical for both 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. As the sample population is identical for both 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 a confounding factor in the study.

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

<table>
<thead>
<tr>
<th>Study (Location)</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>Thorellfsd 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) Sweden 0.316 (T)</td>
<td>G</td>
<td>$3.0 \times 10^{-21}$</td>
<td>20.10 (10.80-37.41)</td>
<td>Iceland 0.153 (A) Sweden 0.121 (A)</td>
</tr>
<tr>
<td>Finget et al$^a$ (USA)</td>
<td>G</td>
<td>.00004</td>
<td>NA</td>
<td>0.400 (T)</td>
<td>G</td>
<td>.00030</td>
<td>NA</td>
<td>0.120 (A)</td>
</tr>
<tr>
<td>Fan et al$^a$ (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>
</tr>
<tr>
<td>Yang et al$^a$ (USA)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.485 (T)</td>
<td>NA</td>
<td>0.147 (A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aragon-Martin et al$^a$ (USA)</td>
<td>G</td>
<td>$7.74 \times 10^{-8}$</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>
</tr>
<tr>
<td>Challa et al$^a$ (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>.0194</td>
<td>3.05 (1.20-7.76)</td>
<td>0.156 (A)</td>
</tr>
<tr>
<td>Hewitt et al$^a$ (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 (1.88-9.02)</td>
<td>NA</td>
</tr>
<tr>
<td>Pasutto et al$^a$ (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>$8.22 \times 10^{-13}$</td>
<td>4.87 (3.46-6.85)</td>
<td>0.149 (A)</td>
</tr>
<tr>
<td>Ramprasad et al$^a$ (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>.0001</td>
<td>4.17 (1.89-9.18)</td>
<td>0.070 (A)</td>
</tr>
<tr>
<td>Lee et al$^a$ (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>.0018</td>
<td>0.97 (1.48-81.49)</td>
<td>0.082 (A)</td>
</tr>
<tr>
<td>Chen et al$^a$ (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>
</tr>
<tr>
<td>Fuse et al$^a$ (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>
</tr>
<tr>
<td>Hayashi et al$^a$ (Japan)</td>
<td>T</td>
<td>$3.0 \times 10^{-13}$</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>
</tr>
<tr>
<td>Ozaki et al$^a$ (Japan)</td>
<td>T</td>
<td>$6.41 \times 10^{-45}$</td>
<td>17.79 (11.03-28.71)</td>
<td>0.497 (G)</td>
<td>G</td>
<td>$1.30 \times 10^{-17}$</td>
<td>10.87 (4.59-25.75)</td>
<td>0.137 (A)</td>
</tr>
<tr>
<td>Williams et al$^a$ (South Africa)</td>
<td>G</td>
<td>$1.7 \times 10^{-5}$</td>
<td>23.2 (3.0-177.2)</td>
<td>0.190 (T)</td>
<td>A</td>
<td>$5.2 \times 10^{-13}$</td>
<td>0.092 (0.045-0.19)</td>
<td>0.380 (A)</td>
</tr>
<tr>
<td>Rautenbach et al (South Africa)$^a$</td>
<td>G</td>
<td>.00106</td>
<td>NA</td>
<td>0.117 (T)</td>
<td>A</td>
<td>$&lt;.00001$</td>
<td>9.94 (4.75-20.79)</td>
<td>0.383 (A)</td>
</tr>
</tbody>
</table>

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

$^a$Present study.

either the genomic environment of LOXL1, the molecular biological environment of elastin fibril metabolism, or the broader environment in which the pathway functions act to increase the complexity of the relationship between LOXL1 and XFS, resulting in these paradoxical associations. Progress in understanding this relationship is dependent on further research in all of these areas.

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 these are not the disease-causing variants but that they are in linkage disequilibrium with the actual pathogenic variants.

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REFERENCES