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Prevalence of Pseudoexfoliation Glaucoma Risk–associated Variants Within Lysyl Oxidase–like 1 in an Irish Population

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Precis: High-risk alleles of risk-associated single-nucleotide polymorphisms (SNPs) within the lysyl oxidase-like 1 (*LOXL1*) gene are associated with pseudoexfoliation in patients recruited from an Irish population.

Purpose: SNPs within the *LOXL1* gene have been identified as a major risk factor for pseudoexfoliation syndrome (PXF) and pseudoexfoliation glaucoma (PXFG), specifically SNPs within exon 1 and intron 1 regions of the gene. The common haplotype (G-G) of 2 SNPs within exon 1, rs1048661, and rs3825942, is the strongest associated risk factor for PXF in white populations, but is switched in some populations to act as protective or low risk. Herein, a study was undertaken to genotype an Irish population for PXF/PXFG risk-associated SNPs within *LOXL1*.

Materials and Methods: Patient cohorts of PXFG, PXF, and controls were recruited and genotyped for risk-associated SNPs within exon 1 (rs1048661 and rs3825942), along with 3 SNPs within intron 1 (rs1550437, rs6495085, and rs6495086) of *LOXL1*.

Results: The risk G alleles of rs1048661 and rs3825942 were most prevalent in PXFG patients, and a significant association was found between rs3825942 and pseudoexfoliation ($P = 0.04$). Genotypes of several intron 1 SNPs were found to be present at higher frequencies within the pseudoexfoliation patient cohort (PXF/PXFG) compared with control patients, wherein rs6495085 showed statistical association ($P = 0.04$). The G-G-G haplotype of rs1048661, rs3825942, and rs6495085 was the most prevalent in PXFG patients compared with control patients or patients with PXF alone. Patients with the G-G-G haplotype were more likely to need surgery, suggestive of a more severe form of disease.

Conclusion: Collectively, these results represent the first study to assess the association of *LOXL1* SNPs with PXFG in an Irish population.

Key Words: pseudoexfoliation glaucoma, lysyl oxidase-like 1, genotyping

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Pseudoexfoliation syndrome (PXF) is an age-related, systemic disorder of the extracellular matrix characterized by a progressive build-up of fibrillar deposits throughout the body.[1] Within ocular tissues, PXF manifests as the accumulation of extracellular matrix/fibrillar materials in the anterior segments of the eye. Moreover, PXF represents a significant risk factor for developing open-angle glaucoma.[2] Progression to pseudoexfoliation glaucoma (PXFG) can occur when the accumulation of fibrillar materials within the trabecular meshwork of the eye leads to reduced aqueous humour outflow and increased intraocular pressure (IOP).[3] The prognosis of PXFG is often worse than that of primary open-angle glaucoma, wherein patients may present with a higher frequency and severity of optic nerve damage at the time of diagnosis, worse visual field damage, a poorer response to medications, a more severe clinical course, and more frequent necessity for surgical intervention.[4] Rates of PXFG vary worldwide, where PXFG was observed in up to 25% of people over 60 in certain Nordic populations.[4,5] Indeed, PXFG is the most common form of glaucoma observed in some countries, including within Ireland.[6,7]

The pathogenesis of PXF and progression to PXFG are still largely unknown and most likely include a combination of genetic and nongenetic components. Genetic studies have indicated that single-nucleotide polymorphisms (SNPs) in the lysyl oxidase-like 1 (*LOXL1*) gene represent a significant risk factor for developing PXF and PXFG.[8] An initial study carried out in a Scandinavian population identified 2 nonsynonymous SNPs within the coding region of *LOXL1*, namely rs1048661 (Leu141Arg) and rs3825942 (Gly153Asp), that were significantly associated with PXF and PXFG risk. Furthermore, the common haplotype (G-G) of these 2 SNPs was found to be the highest associated risk factor for PXF and PXFG.[8] These results were replicated in many more populations.[9–11] However, this risk haplotype was found to be switched in Japanese and South African populations, where the G-G haplotype was observed to be associated with low risk of developing PXF.[12,13] In addition, the intron 1 region of *LOXL1* was subsequently found to contain a promoter that drives expression of a long noncoding RNA, LOXL1-AS1.[14] Several SNPs identified within this region showed a strong association with PXF in 4 independent populations. These SNPs were also found to be functional variants, wherein SNPs were demonstrated to have significant effects on LOXL1-AS1 promoter expression.[14]

Although these SNPs have been genotyped in many populations and Irish patients have been included in several association studies as part of a European cohort,[15] no study has reported on the frequency of *LOXL1* SNPs solely within an Irish population. Therefore, the aim of this study was to recruit patients from an Irish population from 3 distinct

cohorts, those with PXF, PXFG, and controls, and genotype for *LOXL1* SNPs associated with PXF/PXFG risk, specifically focusing on SNPs within exon 1 and intron 1. Risk alleles of all SNPs genotyped were found to be overrepresented in the pseudoexfoliation cohort (PXF/PXFG), while low-risk alleles were mainly found within the control cohort. The exon 1 SNP rs3825942 and the intron 1 SNP rs6495085 were found to be statistically associated with pseudoexfoliation ($P = 0.04$; PXF/PXFG). Furthermore, the G-G-G haplotype constituting the risk alleles of rs1048661, rs3825942, and rs6495085 were found to be most prevalent within the PXFG cohort. PXFG patients with the G-G-G haplotype were also more likely to need surgery, suggestive of a more severe form of disease. Taken together, this study represents an independent evaluation of the prevalence and association of *LOXL1* SNPs in an Irish population.

MATERIALS AND METHODS

Patient Recruitment

Patients were recruited from the Mater Misericordiae University Hospital, Dublin, and Mater Private Hospital, Dublin. The approval from the hospital institutional review board (Ref 1/378/1956) was obtained for the study and was performed according to the tenets of the Declaration of Helsinki. Written informed consent (general data protection regulation compliant) was obtained from all subjects. Patients were subjected to slit-lamp analysis for diagnosis. The diagnosis of PXF (age 71 to 86 y, Table 2) was based on the detection of typical fibrillar white deposits on the lens capsule and pupillary margin. Patients with iris transillumination defects but no fibrillar material deposits were excluded. PXFG (age 63 to 86 y, Table 2) was classified as patients presenting with PXF, along with evidence of glaucomatous changes and visual field loss. Patients with traumatic injury, previous history of intraocular surgery, retinal venous occlusion, age-related macular degeneration, uveitis, and other ocular inflammatory diseases, cardiovascular disease, sepsis, and malignancy, and those with a history of diabetes mellitus and smoking were excluded. The age-matched control cohort (age 66 to 90 y; Table 2) was classified as patients with cataract only and no fibrillar deposits or glaucomatous changes.

Genotyping

Genomic (g) DNA was extracted from whole blood using the GenElute Mammalian Genomic DNA Miniprep kit (Sigma G1N70) as per the manufacturer's instructions. The *LOXL1* SNPs (Table 1) were genotyped in donor gDNA using a double-stranded re-sequencing protocol. The SNP-containing regions of exon 1 (rs1048661 and rs3825942) and intron 1 (LOXL1-AS1 promoter; rs1550437, rs6495085, and rs6495086) were amplified in 2 amplicons by high-throughput polymerase chain reaction. Amplicons were electrophoresed and sequenced on both strands. Data were analyzed manually using the ClustalOmega alignment tool and the Chromas software suite.

Statistical Analysis

Association tests were carried out using PLINK (v1.07 and v2.0)[16] and GraphPad Prism (v6). Because of the small sample size, for all association tests, PXF and PXFG cases were combined to form one pseudoexfoliation cohort. For exploratory single SNP analysis, χ^2 tests were performed, and P values attained were corrected for multiple comparisons using the Bonferroni method ($P = \alpha/n$, where $\alpha = 0.05$ and n = number of tests carried out). Further analysis for significant SNPs was carried out using logistic regression using an additive effects model. P values reported for logistic regression were adjusted for age and sex. Haplotype blocks and linkage disequilibrium (LD) were viewed using Haploview (v4.2). Haplotype association was calculated using a χ^2 test, and P values obtained were, for each individual haplotype, compared with all other haplotypes. Odds ratios for association tests were calculated where possible; however, due to small sample size, some odds ratio values may be inflated.

TABLE 1. *LOXL1* SNPs Genotyped

SNPs	Location*	Alleles	References
rs1048661	Exon 1	G > T	Thorleifsson et al[8]
rs3825942	Exon 1	G > A, G > C, G > T	Thorleifsson et al[8]
rs1550437	Intron 1	C > T	Hauser et al[14]
rs6495085	Intron 1	G > C, G > T	Hauser et al[14]
rs6495086	Intron 1	C > T	Hauser et al[14]
rs1550439†	Intron 1	T > A	—

*Location within the *LOXL1* gene.
†This SNP was not originally included in genotyping experiments but was discovered subsequent to analysis.
LOXL1 indicates lysyl oxidase-like 1; SNP, single-nucleotide polymorphism.

RESULTS

Single SNP Analysis

A total of 23 PXFG, 13 PXF, and 23 control donors were recruited and genotyped for 5 SNPs within the *LOXL1* gene (Table 1). Analysis subsequently uncovered an additional rare variant within intron 1 of *LOXL1* (rs1550439) in our patient cohorts. Clinical features of the patients recruited are outlined in Table 2. There was no significant difference in the sex distribution between the patient cohorts ($P = 0.06$). Patients below 60 years of age were omitted from the study, and no statistical difference was found in the age range of the cohorts. In addition, no association was found between family history of glaucoma and PXF or PXFG (Table 2).

For each of the chosen SNPs, the allele and genotype frequencies were calculated for the control, PXF, and PXFG cohorts (Table 3). The risk alleles of the 2 exon 1 SNPs (rs1048661 and rs3825942) originally reported by Thorleiffsson

TABLE 2. Clinical Features of Patients

	Control (n = 23)	PXF (n = 13)	PXFG (n = 23)	P†
Sex (male/female)	7/16	6/7	15/8	0.06
Age (range)	66-90	71-86	63-86	0.4
Glaucoma medications* (y/n)	—	7/6	21/2	—
Trabeculectomy (y/n)	—	1/12	9/14	—
Laser (y/n)	—	2/11	3/20	—
Family history of glaucoma (y/n)	2/21	4/9	7/16	0.14

*Topical IOP-lowering agents (prostaglandin analogues/beta blockers/carbonic anhydrase inhibitor/pilocarpine/alpha agonists).
†Calculated using either Pearson χ^2 test or Mann-Whitney U test, PXF and PXFG cohorts were combined.
IOP indicates intraocular pressure; PXFG, pseudoexfoliation glaucoma; PXF, pseudoexfoliation syndrome; SNP, single-nucleotide polymorphism.

TABLE 3. Allele and Genotype Frequencies of *LOXL1* SNPs in PXF, PXFG, and Control Cohorts

SNPs	Location	PXFG*	PXF*	Control*
rs1048661	Exon 1			
Allele				
G		36 (0.78)	16 (0.62)	32 (0.70)
T		10 (0.21)	10 (0.38)	14 (0.30)
Genotype				
GG		14 (0.61)	5 (0.38)	15 (0.65)
TG		8 (0.35)	6 (0.46)	2 (0.09)
TT		1 (0.04)	2 (0.15)	6 (0.26)
rs3825942	Exon 1			
Allele				
G		46 (1)	26 (1)	40 (0.86)
A		0	0	6 (0.13)
Genotype				
GG		23 (1)	13 (1)	17 (0.74)
GA		0	0	6 (0.26)
rs1550437	Intron 1			
Allele				
C		44 (0.96)	26 (1)	39 (0.85)
T		2 (0.04)	0	7 (0.15)
Genotype				
CC		21 (0.91)	13 (1)	16 (0.70)
TC		2 (0.09)	0	7 (0.30)
rs6495085	Intron 1			
Allele				
G		46 (1)	26 (1)	40 (0.87)
C		0	0	6 (0.13)
Genotype				
GG		23 (1)	13 (1)	17 (0.24)
CG		0	0	6 (0.26)
rs6495086	Intron 1			
Allele				
C		46 (1)	26 (1)	43 (0.93)
T		0	0	3 (0.07)
Genotype				
CC		23 (1)	13 (1)	20 (0.87)
TC		0	0	3 (0.13)
rs1550439	Intron 1			
Allele				
A		2 (0.04)	1 (0.04)	1 (0.02)
T		44 (0.96)	25 (0.96)	45 (0.98)
Genotype				
AT		2 (0.09)	1 (0.08)	1 (0.04)
TT		21 (0.91)	12 (0.92)	22 (0.96)

*Actual patient (n) numbers are indicated, and allele/genotype frequency is specified within brackets.

LOXL1 indicates lysyl oxidase-like 1; PXFG, pseudoexfoliation glaucoma; PXF, pseudoexfoliation syndrome; SNP, single-nucleotide polymorphism.

and colleagues were found to be most prevalent in patients with PXFG.[10] In contrast to this, there was a higher proportion of the low-risk T allele of rs1048661 in the control cohort compared with PXFG (Table 3). Furthermore, the low-risk A allele and GA genotype of rs3825942 were only identified in the control cohort (Table 3). Low-risk alleles and genotypes of the chosen SNPs within intron 1 were rarely observed, with only 2 of 23 PXFG donors found to be heterozygous for the low-risk TC genotype of rs1550437 (Table 3). All other pseudoexfoliation donors (PXF/PXFG) were found to be homozygous for risk alleles of the 3 intron 1 SNPs chosen for genotyping (rs1550437, rs6495085, and rs6495086; Table 3).

To carry out association analysis, the PXF and PXFG cohorts were combined (pseudoexfoliation cohort). A χ^2 analysis identified 4 SNPs to be significantly associated with pseudoexfoliation (Table 4). Following a Bonferroni correction for multiple comparisons, rs3825942 and rs6495085 maintained association with pseudoexfoliation ($P = 0.002$; Table 4). Further analysis using logistic regression, adjusting for age and sex, indicated that these 2 SNPs remained significantly associated with pseudoexfoliation ($P = 0.04$; Table 4). Conditional analysis of rs3825942 and rs6495085 to test whether the association of each SNP was independent was not possible due to the high LD observed between these 2 SNPs ($r^2 = 1$; Supplementary Fig. 1, Supplemental Digital Content 1, http://links.lww.com/IJG/A367). Although our sample size is small, which may skew r^2 values, these 2 SNPs were previously found to be in LD in a Japanese population.[14]

Haplotype Analysis

Haplotype analysis was carried out, incorporating the 2 exonic SNPs (rs1048661 and rs3825942) and the additional intronic SNP (rs6495085) found to be associated with pseudoexfoliation (PXF/PXFG) in this study. Although rs1048661 was not found to be statistically associated in this study, the risk haplotype it forms with rs3825942 (G-G) is the strongest associated risk factor for pseudoexfoliation in white populations[10] and was therefore included in this haplotype analysis. Of the donors genotyped, 78% of PXFG (Fig. 1) and 62% of PXF (Fig. 1) patients were observed to carry the G-G-G haplotype, corresponding to the risk alleles of each *LOXL1* SNP. Although this haplotype was observed in 57% of the controls, it was the most prevalent within the PXFG cohort. We did not find a statistically significant association between the G-G-G haplotype and the combined pseudoexfoliation cohort (PXF/PXFG) following χ^2 analysis (Table 5). However, when a haplotype association was carried out on the PXFG cohort alone (pseudoexfoliation with glaucoma), the G-G-G haplotype did show a significant association ($P = 0.03$; Supplementary Table 1, Supplemental Digital Content 2, http://links.lww.com/IJG/A368). Furthermore, 14 of the 23 PXFG donors were found to have G-G-G diplotypes, wherein 2 copies of the G-G-G haplotype were observed. The low-risk G-A-C haplotype was only detected in the control donors and not in either the PXFG or PXF cohort and may confer a protective effect (Fig. 1, Table 5).

Finally, we wished to investigate the association, if any, of haplotype with disease severity. To measure disease severity, we looked at whether pseudoexfoliation patients with glaucoma (PXFG) had undergone glaucoma surgery when medications were not controlling the disease. PXFG patients with the G-G-G haplotype were more likely to have undergone surgery compared with those with the T-G-G haplotype (Fig. 2). This association was found to be approaching significance but would require a much larger data set to confirm the association.

DISCUSSION

PXF is currently the single most identifiable risk factor for developing open-angle glaucoma. PXFG patients present with a more severe form of disease, often with a higher IOP and greater levels of optic nerve damage at the time of diagnosis compared with primary open-angle glaucoma. PXFG patients are also clinically harder to manage, as they do not respond as well to medication, require more frequent surgical intervention, and are more likely to suffer from visual impairment and blindness. PXFG has a high incidence in the Irish population and represents up to 60% of glaucoma patients attending glaucoma clinics. Therefore,

TABLE 4. Association Analysis of *LOXL1* SNPs With Pseudoexfoliation (PXF/PXFG) in an Irish Population

SNPs	Location	Risk Allele	Control*	Pseudoexfoliation*†	*P*‡	*P*§	OR‖ (95% CI)
rs1048661	Exon 1	G	0.70	0.72	0.8	0.8	1.1 (0.5-2.6)
rs3825942	Exon 1	G	0.87	1	**0.002**	**0.04**	NA
rs1550437	Intron 1	C	0.85	0.97	0.01	0.04	6.3 (1.2-31.7)
rs6495085	Intron 1	G	0.87	1	**0.002**	**0.04**	NA
rs6495086	Intron 1	C	0.93	1	0.03	0.1	NA
rs1550439	Intron 1	A	0.02	0.04	0.6	0.4	2.0 (0.2-19.4)

NA 0 cell count, OR could not be calculated.
Bold values indicate *P* values that were considered significant after Bonferroni correction and logistic regression.
*Risk allele frequency.
†PXF and PXFG cohorts were combined for association analysis.
‡*P* value obtained by carrying out χ^2 analysis. Bonferroni-corrected significance (0.05/6) is 0.008.
§*P* value obtained by carrying out logistic regression adjusting for sex and age.
‖ORs calculated may be inflated due to small sample size.
CI indicates confidence interval; OR, odds ratio; *LOXL1*, lysyl oxidase-like 1; NA, not applicable; PXFG, pseudoexfoliation glaucoma; PXF, pseudoexfoliation syndrome; SNP, single-nucleotide polymorphism.

identifying and validating risk factors for PXF and PXFG remains an important area of research within the Irish population. Several SNPs in the *LOXL1* gene have been implicated in genetic studies to be a significant risk factor for developing PXF and PXFG.[8,14,17] In the current study, we compared the genotypes of patients from an Irish population who were diagnosed with pseudoexfoliation (PXF/PXFG) with control donors. We then analyzed the association of *LOXL1* SNP genotypes with pseudoexfoliation (PXF/PXFG). Our results show a high proportion of the "risk" alleles within our disease cohorts, wherein 2 of our chosen SNPs were shown to be significantly associated with pseudoexfoliation.

Thorleifsson et al[8] reported that 2 coding SNPs within exon 1 of *LOXL1*, namely rs1048661 (Leu141Arg) and rs3825942 (Gly153Asp), showed the greatest association with PXF and PXFG. Furthermore, the G alleles of these SNPs formed a "high risk" haplotype (G-G), which conferred the greatest risk for PXF and PXFG. This initial study was carried out in a Scandinavian population, where rates of PXF and PXFG are high, and associations have been replicated in many more populations around the world.[9,11,18,19] However, risk alleles and haplotypes have been reported to be switched in Japanese and South African populations,[12,13] indicating regional differences and population-specific effects that must be taken in to account when considering PXF/PXFG risk and *LOXL1* variants. When we examined our disease cohorts, we found the G allele of rs1048661 present in over 78% of PXFG and 62% of PXF donors. Moreover, the risk G allele and high-risk homozygous genotype of rs3824952 was detected in 100% of PXF and PXFG donors. These data correlate with previous studies that report G as the high-risk allele of these 2 SNPs. Although no statistically significant association was found between rs1048661 and pseudoexfoliation, rs3824952 was found to be strongly associated with PXFG ($P = 0.04$; Table 4).

Dysregulation of elastic fiber production and cross-linking is thought to contribute to the etiology of PXF. The gene product of *LOXL1* is an enzyme that catalyzes elastin and collagen cross-linking and is an important mediator of elastin fiber biogenesis and homeostasis. LOXL1 deficiency has been reported in the lamina cribrosa and is thought to contribute to instability in this region, leading to increased susceptibility to optic nerve damage.[20] Expression levels of LOXL1 are altered in ocular tissues during different stages of PXF, wherein LOXL1 was upregulated in the early stages of PXF and downregulated as disease progressed. Furthermore, *LOXL1* mRNA expression is reduced in individuals with the risk GG genotype of rs1048661.[21] The coding variants rs1048861 and rs3825942 may alter protein function and binding, wherein molecular modeling demonstrated that positions 141 (rs1048661) and 153 (rs3825942) of the LOXL1 protein are potential recognition sites for protein-protein interactions.[22] Therefore, changes at these

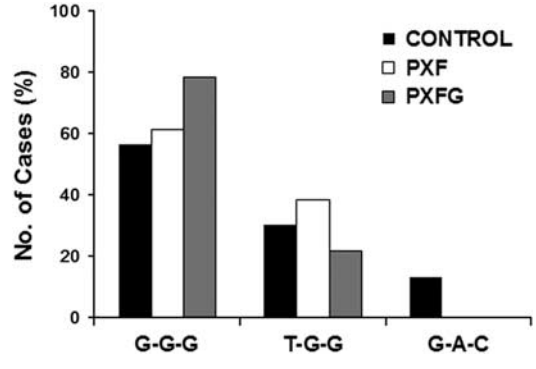

FIGURE 1. Haplotype frequency of *LOXL1* exon 1 variants. Graph depicts the percentage of individuals in each cohort (control, PXF and PXFG) with the G-G-G, T-G-G, and G-A-C haplotypes comprising the 2 coding SNPs rs1048661 and rs3825942 within exon 1 of *LOXL1* and the intronic SNP rs6495085. *LOXL1* indicates lysyl oxidase-like 1; PXFG, pseudoexfoliation glaucoma; PXF, pseudoexfoliation syndrome; SNP, single nucleotide polymorphism.

TABLE 5. Haplotype Analysis of rs1048661, rs3825942, and rs6495085 in Pseudoexfoliation (PXF/PXFG)

	Pseudoexfoliation*				
Haplotypes	PXF	PXFG	Control	*P*	OR† (95% CI)
G-G-G	0.62	0.78	0.57	0.08	2 (0.92-4.36)
T-G-G	0.38	0.22	0.30	0.75	0.9 (0.4-2)
G-A-C	0	0	0.13	0.02	NA

NA 0 cell count, OR could not be calculated.
*For haplotype analysis PXF and PXFG cases were combined.
†ORs calculated may be inflated due to small sample size.
CI indicates confidence interval; OR, odds ratio; NA, not applicable; PXFG, pseudoexfoliation glaucoma; PXF, pseudoexfoliation syndrome.

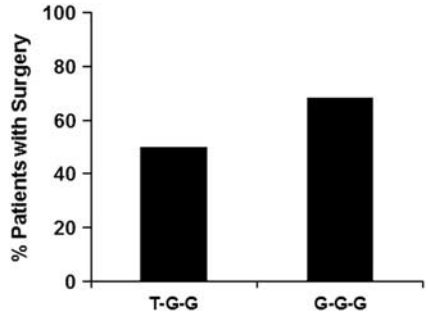

FIGURE 2. Haplotype versus surgery. Graph depicts percentage of PXFG patients with either the T-G-G or G-G-G haplotype who required glaucoma surgery to manage disease. PXFG indicates pseudoexfoliation glaucoma.

residues may alter the ability of LOXL1 to bind to other proteins involved in its cleavage and processing, revealing that PXF-associated SNPs might be directly involved in disease pathogenesis. However, the mechanism whereby genetic variants in *LOXL1* contribute to the development of PXF, and ultimately PXFG, is still poorly understood and will require further research. It is noteworthy that risk alleles of several *LOXL1* SNPs occur in 50% to 90% of the normal population, leading us to believe that other factors such as the environment or epigenetic mechanisms may work in tandem with genetic variants to contribute to disease pathogenesis and progression.[23–26] Indeed, we have found alterations in LOXL1 expression in the Tenon fibroblasts and aqueous humour taken from PXFG patients. Moreover, these alterations are mimicked in Tenon's fibroblasts from control patients under hypoxic and oxidative stress conditions, indicating a strong environmental influence (unpublished data). Although genetic studies alone may be limited, they represent an important step in deducing population-specific risk for PXF/PXFG.

Although ~10% of SNPs associated with disease are located within coding regions, over half are found in non-coding regions.[27] Several SNPs within intron 1 of *LOXL1* were also found to be associated with PXF/PXFG risk and have functional effects on promoter activity of a long non-coding (lnc) RNA, LOXL1-AS1.[14] LncRNAs are non-coding RNAs >200 nucleotides. They are now understood to have important roles in regulating gene expression and can act in both a *cis* and *trans* manner.[28] LncRNAs have also been shown to be involved in the pathogenesis of diseases such as cancer and cardiovascular disease.[29,30] Specifically, LOXL1-AS1 has been reported to be involved in cancer cell proliferation, migration, and invasion in several forms of cancer.[31,32] In terms of PXFG, it is suggested that LOXL1-AS1 plays a role in responding to environmental stimuli such as oxidative stress.[14] In this study, we genotyped our patient cohorts for 3 SNPs, rs1550437, rs6495085, and rs6495086, within intron 1 of *LOXL1* previously reported by Hauser et al.[14] We found risk alleles for each of these SNPs to be overrepresented in disease cohorts. Moreover, 1 SNP within intron 1, rs6495085, was found to be significantly associated with PXFG ($P = 0.04$; Table 4). This study, to the best of our knowledge, represents the first to report the frequency of these SNPs within an Irish population. In addition, our re-sequencing of this region of *LOXL1* in our cohorts also captured a rare intron 1 SNP, rs1550439. There are few clinical data and/or population studies published on this SNP with regard to PXF/PXFG risk, and we did not find any significant association with PXF or PXFG.

The haplotype analysis of rs1048661, rs3825942, and rs6495085 demonstrated that the "high risk" G-G-G haplotype was most prevalent in the PXFG cohort, while the "low risk" G-A-C haplotype was only observed in control donors. In addition, patients with high risk G-G-G haplotype were more likely to need glaucoma surgery, suggesting that they suffered from a more severe form of disease. Typically, drugs are administered to lower/control the IOP of the patient and therefore reduce the risk for optic nerve damage and vision loss. However, if IOP-lowering agents are failing, surgical intervention is needed. In this study, we observed that a higher proportion of patients with the G-G-G haplotype underwent glaucoma surgery.

In conclusion, we have demonstrated that the *LOXL1* SNPs rs3825942 and rs6495085 are associated with pseudoexfoliation in patients recruited from an Irish population. Although we found no significant association between rs1048661 and PXF/PXFG, we report an association between the high-risk G-G-G haplotype of rs1048661, rs3825942, and rs6495085 with PXFG. Furthermore, we found that patients with the G-G-G haplotype were more likely to need surgery, suggestive of a more severe form of the disease. We acknowledge that the sample size of this study is small, limiting its ability to find true association. However, we have clearly replicated previous frequency and associations of the chosen SNPs in our study. Given the prevalence of PXFG within the Irish population and the strong genetic link between *LOXL1* and PXFG, this study represents an important milestone in determining the genetic risk of developing the disease. However, current knowledge indicates that genetics may only be part of the risk for developing PXF and PXFG; therefore, future studies into the interplay between genetic, epigenetic, and environmental factors are warranted.

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