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ORIGINAL ARTICLE

Association of *LOXL1* gene with Finnish exfoliation syndrome patients

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In this study, three single-nucleotide polymorphisms (SNPs) on the lysyl oxidase-like 1 (*LOXL1*) gene associated with exfoliation syndrome (XFS) and exfoliation glaucoma (XFG) were investigated in the Finnish population. A case–control study of 59 sporadic patients with XFS, 82 with XFG, 71 with primary open-angle glaucoma (POAG) and 26 individuals without these disorders from the southern Finnish population, and a family study of an extended family with 28 patients with XFS or XFG and 92 unaffected relatives from Kökar islands, Southwestern Finnish archipelago, were conducted. Anonymous blood donors (n=404) were studied as population-based controls. Three SNPs, rs1048661 (R141L), rs3825942 (G153D) and rs2165241, of the *LOXL1* gene were genotyped by PCR sequencing. Association and linkage analyses were carried out. In both case–control and family materials, significant association for allele G of rs1048661 ($P=2.65\times10^{-5}$; $P=0.0007$), allele G of rs3825942 ($P=2.24\times10^{-8}$; $P=0.49$) and allele T of rs2165241 ($P=2.62\times10^{-13}$; $P<0.0001$) was found in XFS/XFG. However, linkage was not observed for *LOXL1* risk alleles. The corresponding three-locus haplotype GGT increased the risk of XFS/ XFG nearly 15-fold relative to low-risk haplotype GAC (odds ratio (OR): 14.9, $P=1.6\times10^{-16}$). In conclusion, the earlier reported polymorphisms of the *LOXL1* gene showed significant association also in the Finnish population.
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INTRODUCTION

Exfoliation syndrome (XFS) is an age-related accumulation of microfibrillar extracellular deposits with associated extracellular matrix in the anterior segment of the eye, most commonly seen in the pupillary border of the iris and on the anterior lens capsule. Similar fibrils have also been detected in extraocular tissues. XFS is a major risk factor for development and progression of exfoliation glaucoma (XFG).[1] Approximately one quarter of XFS patients have elevated intraocular pressure and one-third of these have XFG.[2–4] XFS and XFG occur worldwide, but their prevalence varies between different populations. The average worldwide prevalence ranges from 5 to 20% of the population older than 60 years of age.[5] In Finland, the prevalence of XFS is 22% among individuals 70 years of age and older.[6]

Familial aggregation and twin studies suggested genetic contribution to XFS.[7–10] A genome-wide association study in the Icelandic population showed three single-nucleotide polymorphisms (SNPs), rs1048661 (R141L), rs3825942 (G153D) and rs2165241, on lysyl oxidase-like 1 (*LOXL1*) gene, on 15q24.1, to be strongly associated with XFS and XFG, but not with primary open-angle glaucoma (POAG).[11] The association between *LOXL1* polymorphisms and XFG was further confirmed in the Swedish population. Taken together, two non-synonymous SNPs, rs1048661 and rs3825942, accounted for 99% of the population-attributable risk (PAR) of XFG in the combined cohort. Three of the four possible haplotypes of coding SNPs were observed, of which GG and TG were considered risk haplotypes relative to GA.

The *LOXL1* gene, a member of the lysyl oxidase gene family, is expressed in various ocular tissues. The product of *LOXL1* gene, lysyl oxidase-like protein 1, modifies elastin fibers, which are a major constituent of intraocular exfoliation material. Hence, *LOXL1* potentially contributes to the pathogenesis of the exfoliation syndrome.

We investigated the role of the three *LOXL1* polymorphisms in the Finnish population using a case–control material and an extended family from an island in the southwestern Finnish archipelago, in which XFS shows autosomal dominant inheritance with incomplete penetrance.[12]

MATERIALS AND METHODS

Patient material

The case–control material consisted of 141 patients with sporadic XFS with or without XFG on the one hand, and 71 patients with POAG and 26 individuals with no evidence of ocular disease on the other (mean age 86 years, range:

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Table 1 Number of patients and controls in the study

Diagnosis	*Sporadic*	*Familial*	*Total*
XFS	59	19	78
XFG	82	9	92
POAG	71	—	71
Unaffected (all ages)		92	92
Unaffected (>80 years)	26		26
Blood donor controls			404

Abbreviations: POAG, primary open-angle glaucoma; XFG, exfoliation glaucoma; XFS, exfoliation syndrome.

81–93 years), all from Tammisaari parish in southern Finland (Table 1). In addition, 404 unexamined anonymous Finnish blood donors from three different parishes, Salo (n=121), Lieksa (n=108) and Kurikka (n=175), were used as population-based controls. The extended family from Kökar island in the southwestern Finnish archipelago included 28 family members affected with XFS, with or without XFG, and 92 unaffected relatives (mean age 63 years, range: 32–92 years).[12]

The diagnoses are based on a comprehensive ophthalmic examination, including both slit-lamp biomicroscopy and funduscopy, before and after dilatation. XFS was recorded when a grayish central disc, with or without a peripheral band, on the anterior lens capsule or fibrillary material on the pupillary ruff was detected. A patient was recorded as affected if XFS was detected in at least one eye. Krukenberg's spindle, pigment dispersion and diffuse haze on the anterior capsule, without the signs mentioned above, were coded as XFS negative. The recorded age was the age when XFS was first observed or, if XFS was not present, the age at the last examination.

Diagnosis of glaucoma was on the basis of a combination of structural changes in the optic disc (cup-to-disc ratio $\geqslant$0.7, localized thinning of the rim, asymmetry $\geqslant$0.2 between the discs) and glaucomatous visual field defect, with or without intraocular pressure $\geqslant$22 mm Hg, in the presence of open anterior chamber angle. Glaucoma without XFS was recorded as POAG.

The study was approved by the institutional review boards of the Department of Ophthalmology, Helsinki University Central Hospital and Central Hospital of Åland, Mariehamn, Finland, and was conducted in accordance with the Declaration of Helsinki. The participants gave written informed consent.

Laboratory procedures

EDTA blood samples were collected from all participants. Genomic DNA was extracted from the peripheral blood with the standard phenol–chloroform procedure. Two coding SNPs on exon 1, rs1048661 (R141L) and rs3825942 (G153D), and one non-coding SNP on intron 1, rs2165241, of *LOXL1* gene were genotyped by direct sequencing. Three SNPs were sequenced in two PCRs (exonic SNPs in one reaction and intronic in the other) as described elsewhere.[13] The primer sequences are available on request from the authors.

Statistical analyses

Case–control material. Hardy–Weinberg equilibrium was tested separately in patients and controls using χ^2-test (with 5% level of significance). Allele, genotype and haplotype frequency differences between POAG or XFS or XFG patients and controls were estimated by Pearson's χ^2-test with Yates' continuity correction (in R program). The odds ratios (ORs) with 95% confidence intervals and Fisher's exact P-values were estimated using R package epitools. PARs (%) were estimated using Levin's formula. The haplotypes were reconstructed using SNPHAP 1.2.1. Only those haplotypes showing >90% reliability by SNPHAP 1.2.1 were accepted for further analyses.

Family material. The Mendelian inheritance in the pedigree was assessed using Pedcheck 1.2.1.[14] Association was measured by the χ^2-test using independent cases and controls picked from the pedigrees. The haplotypes were reconstructed with FBAT 2.0.2c.[15,16] Linkage analysis was carried out on *LOXL1* SNP genotype data, combined with chromosome 15 microsatellite genotype data from an earlier genome-wide scan.[12] Linkage analysis was carried out in 64 participants from pedigree; of these, 28 were XFS/XFG affected and 36 were unaffected.[12] Two-point linkage analyses under both homogeneity and heterogeneity model were carried out using the MLINK program of the LINKAGE package.[17,18] The marker allele frequencies were estimated using the DOWNFREQ 2.1 program.[19] Tests for heterogeneity and calculations of proportion of linked families (α) were carried out using the HOMOG 3.35 program.[20] The disease model for linkage analysis was a dominant (dom) model with low penetrance and phenocopy rate (0.08, 0.5 and 0.5) and common disease allele frequency (0.45). Clinically unaffected family members were scored as unaffected, because their mean age at the time of the last examination was 75.3 (range, 51–94), and because the alternative, the affected-only method, would have led to substantial loss of information. Individuals belonging to the pedigree without genotype data and without XFS and XFG diagnosis were scored as unknown. The region containing the *LOXL1* gene was further examined by multipoint analysis using the Superlink 1.6 program.[21,22]

RESULTS

Association in the case–control material

LOXL1 alleles and genotypes in the case–control study. All three *LOXL1* SNPs were significantly associated with XFS and XFG, but not with POAG, when compared with blood donor controls. The strongest allelic association was observed with allele T of intronic SNP rs2165241 (χ^2-test, combined cohort, $P=2.62\times10^{-13}$). Allele G of the exonic SNPs rs1048661 (χ^2-test, $P=2.65\times10^{-5}$) and allele G of exonic SNP rs3825942 (χ^2-test, $P=2.24\times10^{-8}$) were also significantly associated with XFS and XFG. The allele G of coding SNP rs3825942 produced the highest OR, 6.43 ($P=4.82\times10^{-10}$) (Table 2). No deviation from Hardy–Weinberg equilibrium was found in any loci in the controls.

When genotypes were considered, genotype TT of the intronic SNP rs2165241 showed the strongest association with XFS and XFG (χ^2-test, $P=6.92\times10^{-11}$). Both coding SNP genotypes GG of rs1048661 and rs3825942 were preferentially present in the combined exfoliation cohort (χ^2-test, $P=5.61\times10^{-5}$ and 8.88×10^{-8}, respectively) (Table 3). PARs for allele G of rs1048661 and rs3825942 and allele T of rs2165241 were 45, 82 and 50%, respectively, and the corresponding genotype PARs were 50, 79 and 62%, respectively.

LOXL1 haplotypes in the case–control study. The two-locus haplotype, GG, composed of coding SNPs rs1048661 and rs3825942, was overrepresented in XFS and XFG cases (χ^2–test, $P=9.11\times10^{-16}$) but not in POAG cases (χ^2-test, P=0.98), as compared with blood donor controls (Table 4). Approximately 95% of XFS and XFG patients carried at least one GG haplotype and of them 70% were GG homozygous, whereas 72% of blood donor controls had GG haplotype, of which 40% were in homozygous form. The PAR value for the high-risk haplotype GG was 60%. Both TG and GA haplotypes were underrepresented in XFS and XFG cases compared with the blood donor controls (χ^2-test, $P=1.61\times10^{-5}$ and 4.32×10^{-9}, respectively). However, one XFS patient homozygous for GA haplotype was identified (Table 4). A comparison of the GG and TG haplotypes relative to the GA haplotype in XFS and XFG cases yielded ORs of 9.37 ($P=2.46\times10^{-14}$) and 3.16 (P=0.002), respectively (Figure 1). Haplotype TA was observed neither in the cases nor in the controls, with one possible exception of a case diagnosed with POAG, although this might have resulted from an error in the haplotype reconstruction.

When the combined effects of all three SNPs are taken into consideration, the haplotype GGT is highly overrepresented in XFS and XFG cases (χ^2-test, $P=5.97\times10^{-14}$), but not in POAG cases (χ^2-test, P=0.98) (Table 5). The GGT haplotype was present in 93% of XFS and XFG patients (of them 59% were GGT homozygous) and in 69% of blood donor controls (34% homozygous). The PAR value of the GGT haplotype was 52%. Both TGC and GAC haplotypes were

Table 2 Risk-allele counts and frequencies for three *LOXL1* SNPs, and their corresponding association test between exfoliation syndrome, exfoliation glaucoma, primary open-angle glaucoma and controls

	rs1048661 G (R141L)			*rs3825942G (G153D)*			*rs2165241 T*		
Study groups (n)	*Freq. (counts)*	*OR (95% CI)*	P-*value*	*Freq. (counts)*	*OR (95% CI)*	P-*value*	*Freq. (counts)*	*OR (95% CI)*	P-*value*
Finnish case–control material									
Exfoliation combined (141)	0.83 (208/252)	2.19 (1.53–3.18)	1.47×10^{-5}	0.97 (244/252)	6.43 (3.29–14.60)	4.82×10^{-10}	0.73 (205/280)	3.10 (2.28–4.23)	7.36×10^{-14}
XFS (59)	0.82 (82/100)	2.10 (1.25–3.40)	0.0048	0.94 (94/100)	3.29 (1.52–8.67)	0.0020	0.72 (85/118)	2.91 (1.91–4.54)	4.12×10^{-7}
XFG (82)	0.83 (126/152)	2.24 (1.44–3.59)	0.00032	0.99 (150/152)	14.97 (4.69–98.14)	2.43×10^{-9}	0.74 (120/162)	3.23 (2.22–4.79)	4.12×10^{-10}
POAG (71)	0.69 (93/134)	1.05 (0.71–1.59)	0.84	0.79 (107/136)	0.79 (0.51 −1.27)	0.33	0.46 (64/140)	0.96 (0.66–1.38)	0.85
Blood donor controls (404)	0.68 (444/650)			0.82 (535/650)			0.47 (296/632)		

Abbreviations: freq., frequency; OR (95% CI), odds ratio (95% confidence interval); POAG, primary open-angle glaucoma; SNP, single-nucleotide polymorphism; XFG, exfoliation glaucoma; XFS, exfoliation syndrome.

Table 3 Genotypic counts and frequencies for three *LOXL1* SNPs, and their corresponding association test between exfoliation syndrome, exfoliation glaucoma, primary open-angle glaucoma and controls

	LOXL1 SNP genotypes		
	rs1048661 (R141L)	*rs3825942 (G153D)*	*rs2165241*
Study groups (n)	*GG/GT/TT*	*GG/GA/AA*	*TT/TC/CC*
Exfoliation combined (141)			
Counts	(88/32/6)	(119/6/1)	(76/53/11)
Freq	0.70/0.25/0.05	0.94/0.05/0.008	0.54/0.38/0.08
P-value	0.01	0.02	6.27×10^{-12}
OR (95% CI)[a]	3.11 (1.33–8.62)	6.55 (1.29–160.28)	8.86 (4.50–18.97)
XFS (59)			
Counts	34/14/2	45/4/1	32/21/6
Freq	0.68/0.28/0.04	0.90/0.08/0.02	0.54/0.36/0.10
P-value	0.08	0.48	7.05×10^{-6}
OR (95% CI)[a]	3.44 (0.97–23.74)	2.48 (0.48–61.40)	6.78 (2.83–19.12)
XFG (82)			
Counts	54/18/4	74/2/0	44/32/5
Freq	0.71/0.24/0.05	0.97/0.03/0	0.54/0.40/0.06
P-value	0.06	0.13	5.30×10^{-9}
OR (95% CI)[a]	2.83 (1.05–10.06)	4.08 (0.80–100.18)	11.10 (4.51–34.03)
POAG (71)			
Counts	32/29/6	41/25/2	17/30/23
Freq	0.48/0.43/0.09	0.60/0.37/0.03	0.24/0.43/0.33
P-value	1.00	1.00	1.00
OR (95% CI)[a]	1.14 (0.46–3.26)	1.21 (0.32–8.59)	0.97 (0.47–1.96)
Blood donor controls (404)			
Counts	152/140/33	224/87/14	65/166/85
Freq	0.47/0.43/0.10	0.69/0.27/0.04	0.21/0.53/0.27

Abbreviations: freq., frequency; OR (95% CI), odds ratio (95% confidence interval); POAG, primary open-angle glaucoma; SNP, single-nucleotide polymorphism; XFG, exfoliation glaucoma; XFS, exfoliation syndrome.
[a]The odds ratios are given for the high-risk homozygous genotype versus the low-risk homozygous genotype.

underrepresented in the XFS and XFG group compared with the blood donor controls (χ^2-test, $P=2.79\times10^{-5}$ and $P=2.19\times10^{-10}$, respectively). Again, one of the aforementioned XFS patients was found to be homozygous for the low-risk haplotype GAC. When haplotypes GGT and TGC were compared with low-risk haplotype GAC, OR of 14.91 ($P=1.67\times10^{-16}$) and 4.98 ($P=0.00018$), respectively, were obtained (Figure 1). Haplotypes GGC, TGT and GAT were not significantly differently represented in the cases and controls.

Table 4 Estimated two-loci haplotype frequencies for *LOXL1* SNPs rs1048661, rs3825942 and rs2165241, and their corresponding association test between exfoliation syndrome, exfoliation glaucoma, primary open-angle glaucoma and controls

rs1048661	*rs3825942*		*XFS* (n=*59*)	*XFG* (n=*82*)	*Exfoliation combined* (n=*141*)	*POAG* (n=*71*)	*Controls (blood donors,* n=*404*)
G	G	Freq	0.78	0.82	0.80	0.50	0.51
		P-value	1.42×10^{-7}	3.67×10^{-13}	1.12×10^{-16}	0.92	
		OR (95% CI)[a]	3.40 (2.13–5.63)	4.45 (2.90–7.04)	3.97 (2.83–5.67)	0.98 (0.67–1.42)	
		PAR	55.04	63.76	60.23	−1.03	
T	G	Freq	0.17	0.17	0.17	0.29	0.31
		P-value	0.0024	0.00026	9.07×10^{-06}	0.68	
		OR (95% CI)[a]	0.44 (0.25–0.74)	0.44 (0.27–0.69)	0.44 (0.30–0.64)	0.90 (0.59–1.35)	
		PAR	−21.01	−21.01	−21.01	−0.31	
G	A	Freq	0.06	0.01	0.03	0.20	0.18
		P-value	0.00052	7.45×10^{-10}	8.82×10^{-11}	0.62	
		OR (95% CI)[a]	0.27 (0.10–0.59)	0.062 (0.01–0.20)	0.14 (0.06–0.28)	1.14 (0.71–1.81)	
		PAR	−15.13	−20.37	−18.32	2.46	
T	A	Freq	0.00	0.00	0.00	0.007	0.00
		P-value					
		OR (95% CI)[a]					
		PAR					

rs1048661	*rs2165241*		*XFS* (n=*59*)	*XFG* (n=*82*)	*Exfoliation combined* (n=*141*)	*POAG* (n=*71*)	*Controls (blood donors,* n=*404*)
G	T	Freq	0.77	0.74	0.75	0.46	0.46
		P-value	3.67×10^{-9}	3.87×10^{-10}	2.28×10^{-15}	0.92	
		OR (95% CI)[a]	3.85 (2.42–6.32)	3.37 (2.28–5.06)	3.56 (2.58–4.96)	0.98 (0.67–1.43)	
		PAR	56.73	52.16	54.08	−0.93	
T	C	Freq	0.17	0.16	0.16	0.29	0.30
		P-value	0.0034	0.00033	1.66×10^{-5}	0.84	
		OR (95% CI)[a]	0.46 (0.26–0.78)	0.44 (0.27–0.69)	0.45 (0.31–0.65)	0.94 (0.62–1.41)	
		PAR	−19.3	−20.19	−19.76	−1.83	
G	C	Freq	0.06	0.08	0.08	0.25	0.23
		P-value	3.69×10^{-5}	8.69×10^{-5}	9.21×10^{-8}	0.65	
		OR (95% CI)[a]	0.24 (0.10–0.50)	0.34 (0.18–0.59)	0.30 (0.18–0.48)	1.12 (0.72–1.72)	
		PAR	−27.72	−26.78	−26.78	−20.78	
T	T	Freq	0	0.006	0.004	0.007	0.009
		P-value	1	1	0.67	1	
		OR (95% CI)[a]	1.12 (0.04–7.35)	0.78 (0.03–9 5.11)	0.46 (0.02–3.00)	0.88 (0.03–5.78)	
		PAR	0.108	−0.198	−0.49	−0.11	

rs3825942	*rs2165241*		*XFS* (n=*59*)	*XFG* (n=*82*)	*Exfoliation combined* (n=*141*)	*POAG* (n=*71*)	*Controls (blood donors,* n=*404*)
G	T	Freq	0.74	0.75	0.75	0.46	0.47
		P-value	1.80×10^{-7}	3.78×10^{-10}	4.81×10^{-14}	0.85	
		OR (95% CI)[a]	3.22 (2.05–5.19)	3.38 (2.29–5.10)	3.32 (2.41–4.62)	0.95 (0.65–1.38)	
		PAR	51.15	52.76	52.17	−2.41	
G	C	Freq	0.20	0.24	0.22	0.33	0.35
		P-value	0.003	0.009	0.0003	0.76	
		OR (95% CI)[a]	0.48 (0.28–0.78)	0.58 (0.38–0.87)	0.54 (0.38–0.75)	0.94 (0.63–1.38)	
		PAR	−22.25	−17.23	−19.19	−21.45	
A	C	Freq	0.03	0.01	0.02	0.20	0.18
		P-value	8.14×10^{-6}	1.32×10^{-9}	4.96×10^{-13}	0.54	
		OR (95% CI)[a]	0.14 (0.03–0.37)	0.06 (0.01–0.20)	0.09 (0.03–0.20)	1.16 (0.71–1.83)	
		PAR	−18.32	−20.39	−19.5	2.80	
A	T	Freq	0.03	0.00	0.01	0.00	0.002
		P-value	0.015		0.10		
		OR (95% CI)[a]	14.27 (1.64–411.64)		5.76 (0.67–165.75)		
		PAR	2.59		0.94		

Abbreviations: freq., frequency; OR (95% CI), odds ratio (95% confidence interval); SNP, single-nucleotide polymorphism; PAR, population-attributable risk; POAG, primary open-angle glaucoma; XFG, exfoliation glaucoma; XFS, exfoliation syndrome.
[a]The odds ratios and *P*-values were calculated for each individual haplotype compared with all the other haplotypes.

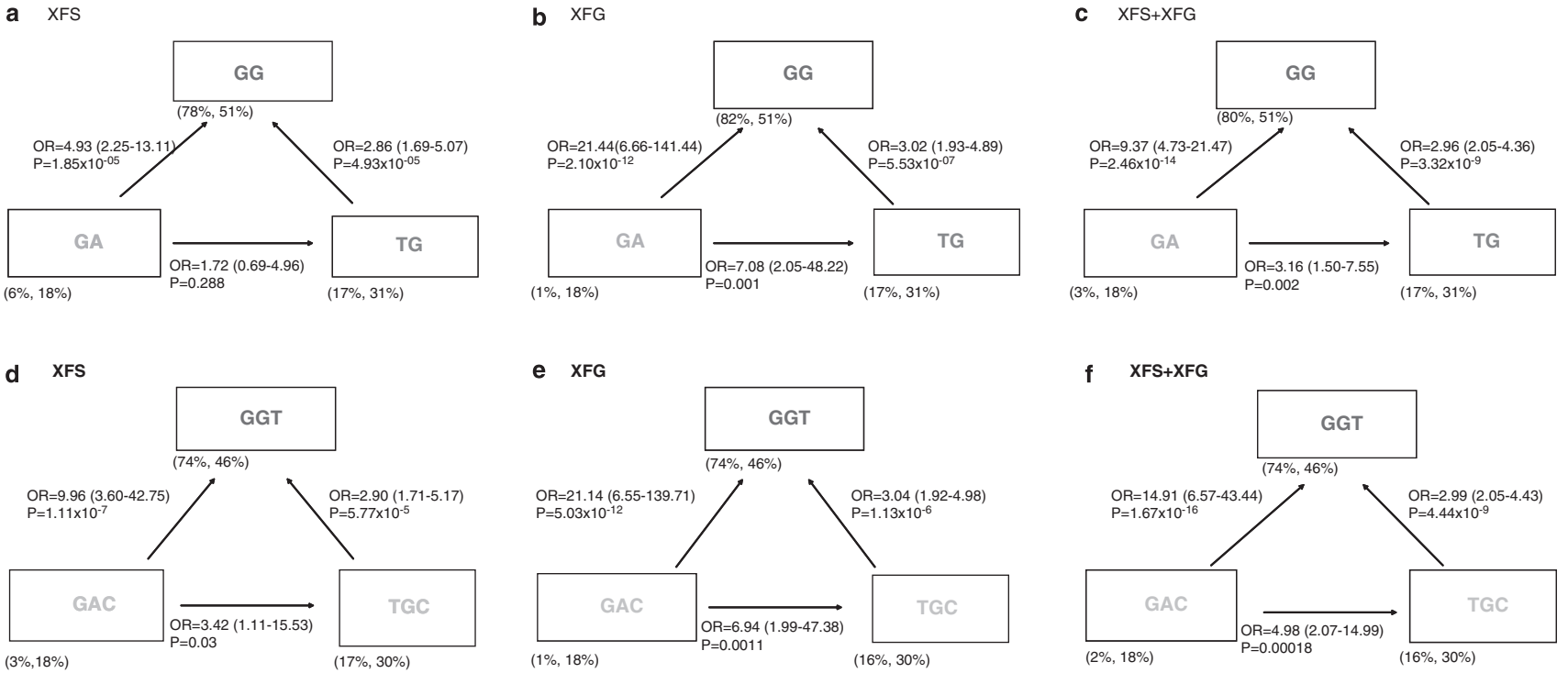

Figure 1 Association of two-locus haplotypes GG, TG and GA formed by single-nucleotide polymorphisms rs1048661 and rs3825942 with (**a**) exfoliation syndrome (XFS), (**b**) exfoliation glaucoma (XFG) and the (**c**) combined exfoliation cohort, and that of three-locus haplotypes GGT, TGC and GAC formed by SNPs rs1048661, rs3825942 and rs2165241 with (**d**) XFS, (**e**) XFG and the (**f**) combined exfoliation cohort. The arrows depict pairwise comparisons between the haplotype boxes. Haplotype frequencies in cases and controls are shown in parentheses below each haplotype box.

Table 5 Estimated three-loci haplotype frequencies for *LOXL1* SNPs, and their corresponding association test between exfoliation syndrome, exfoliation glaucoma, primary open-angle glaucoma and controls

Three-loci haplotypes	*XFS (n=59)*	*XFG (n=82)*	*Exfoliation combined (n=141)*	*POAG (n=71)*	*Controls (blood donors, n=404)*
GGT					
Freq	0.74	0.74	0.74	0.46	0.46
P-value	9.02×10^{-8}	2.32×10^{-10}	1.66×10^{-14}	1	
OR (95% CI)[a]	3.34 (2.13–5.39)	3.39 (2.30–5.10)	3.38 (2.45–4.69)	0.99 (0.68–1.44)	
PAR	51.84	52.37	52.26	−0.46	
TGC					
Freq	0.17	0.16	0.16	0.29	0.30
P-value	0.0034	0.00033	1.66×10^{-5}	0.84	
OR (95% CI)[a]	0.46 (0.26–0.78)	0.44 (0.27–0.69)	0.45 (0.31–0.65)	0.94 (0.62–1.41)	
PAR	−19.33	−20.19	−19.76	−1.83	
GAC					
Freq	0.03	0.01	0.02	0.20	0.18
P-value	8.14×10^{-6}	1.32×10^{-9}	4.96×10^{-13}	0.54	
OR (95% CI)[a]	0.14 (0.03–0.37)	0.06 (0.01–0.20)	0.09 (0.03–0.20)	1.16 (0.71–1.83)	
PAR	−18.32	−20.37	−19.59	2.80	
GGC					
Freq	0.04	0.08	0.06	0.04	0.05
P-value	1.00	0.15	0.39	1.00	
OR (95% CI)[a]	0.83 (0.23–2.21)	1.75 (0.83–3.52)	1.35 (0.69–2.57)	0.97 (0.35–2.28)	
PAR	−0.86	3.61	1.69	−0.15	
TGT					
Freq	0.00	0.006	0.004	0.00	0.009
P-value	1.00	1.00	0.67		
OR (95% CI)[a]	1.12 (0.04–7.35)	0.78 (0.03–5.11)	0.46 (0.02–3.00)		
PAR	0.108	−0.198	−0.49		
GAT					
Freq	0.03	0.00	0.01	0.00	0.002
P-value	0.02		0.10		
OR (95% CI)[a]	14.27 (1.64–411.64)		5.76 (0.67–165.75)		
PAR	2.58		0.94		
TAT					
Freq	0.00	0.00	0.00	0.007	0.00
P-value					
OR (95% CI)[a]					
PAR					
TAC					
Freq	0.00	0.00	0.00	0.00	0.00
P-value					
OR (95% CI)[a]					
PAR					

Abbreviations: freq., frequency; OR (95% CI), odds ratio (95% confidence interval); SNP, single-nucleotide polymorphism; PAR, population-attributable risk; POAG, primary open-angle glaucoma; XFG, exfoliation glaucoma; XFS, exfoliation syndrome.
[a]The odds ratios and *P*-values were calculated for each individual haplotype compared with all the other haplotypes.

Haplotypes TAC and TAT were not observed, with, again, the possible exception of the single POAG case mentioned above.

Association and linkage in the family material

In the original genome-wide scan in our XFS family, the area around the *LOXL1* gene showed a suggestive logarithm of odds (LOD) score of 1.19 (θ=0.10, α=1.00), with marker D15S1032 at region 15q21.2.[12] This marker is located ~21 cM away from the SNPs on the *LOXL1* gene. However, the nearest markers of the *LOXL1* gene, D15S145 and D15S114, did not show any hint of linkage. When we combined the *LOXL1* SNP genotype data with the genome scan microsatellite genotype data of chromosome 15 and re-calculated the two-point

Table 6 Three-loci haplotype frequencies in the familial and case–control material

	Familial		*Sporadic*		
Haplotype	*Exfoliation combined (n=28)*	*Unaffected (n=92)*	*Exfoliation combined (n=141)*	*Unaffected (n=26)*	*Blood donors (n=404)*
GGT	0.80	0.43	0.74	0.35	0.46
TGC	0.16	0.48	0.16	0.38	0.30
GAC	0.03	0.07	0.02	0.19	0.18
TGT	0.01	0.009	0.004	—	0.01
GGC	—	0.02	0.06	0.08	0.05
GAT	—	—	0.01		0.002
TAC	—	—	—		—
TAT	—	—	—		—

Table 7 Allele and haplotype associations in the family material

	rs1048661 G (R141L)	*rs3825942 G (G153D)*	*rs2165241 T*	*Combined haplotypes: GGT vs all other haplotypes*
Frequencies				
Exfoliation combined	0.79	0.98	0.79	0.77
Unaffected	0.45	0.93	0.40	0.38
χ^2-test statistic[a]	χ^2=11.55	χ^2=0.49	χ^2=15.21	χ^2=14.77
P-value	*P*=0.0007		*P*<0.0001	*P*=0.0001

[a]Association is measured by the χ^2-test using independent cases and controls picked from the pedigrees.

and multipoint linkage, no significant two- or multipoint LOD scores were obtained with any of the three *LOXL1* SNPs, nor with the surrounding markers (data not shown). However, allelic and haplotype distributions in XFS and XFG patients and controls were similar both in the family and in the case–control material. Of the three-locus haplotypes, only GGT haplotype was slightly more prevalent in the family sample set (0.80 in the cases and 0.43 in the examined controls) than in the case–control material (0.74 in the cases and 0.35 in the examined controls) (Table 6). Association was measured in the family material using independent cases and controls picked from the pedigrees. Allele G of exonic SNP rs1048661 (χ^2-test, P=0.0007) and allele T of intronic SNP rs2165241 (χ^2-test, $P<0.0001$) were preferentially present in the combined exfoliation cohort (Table 7). Association for allele G of exonic SNP rs3825942 was not observed, because of its high frequency both in exfoliation patients and in unaffected relatives (0.98 versus 0.93, χ^2-test P=0.49). However, the three-locus haplotype GGT showed a strong association with XFS and XFG in the family (χ^2-test, P=0.0001).

DISCUSSION

Our results confirm a strong association between the three *LOXL1* SNPs and exfoliation also in the Finnish population. This is in agreement with the earlier studies in other Caucasian populations (Scandinavians,[11] and other European[23–26] and American populations[25,27–29]), in the Australian population[30] and also in African-Americans[26] and in the Asian populations from Japan[31–35] and India.[36] The strongest allelic effect in the Finnish case–control material was found for allele G of exonic SNP rs3825942, which confers the greatest risk in all populations studied so far. Allele of intronic SNP rs2165241 and allele G of exonic SNP rs1048661 were also significantly associated with XFS and XFG. In the genotype comparisons, genotype TT of the intronic SNP rs2165241 showed the strongest association with XFS and XFG, but genotypes GG of rs1048661 and rs3825942 were also significantly associated.

The two-locus haplotype GG of coding SNPs rs1048661–rs3825942 was highly overrepresented, whereas TG and GA haplotypes were underrepresented in XFS and XFG cases compared with the population-based controls, which is convergent with earlier studies of Caucasian populations.[11,23–25] The high-risk haplotype GG was present in 95% of the Finnish exfoliation cases and two copies of GG haplotype (GG homozygosity) were identified in 66% of patients. The same proportion of GG homozygotes was reported earlier in German and Italian exfoliation patients.[24] Together GG and TG haplotypes accounted for 97% of the exfoliation cases, being in agreement with the corresponding results in Scandinavian (99%) and other European populations (96%).[11,25] On the other hand, the high-risk haplotype GG is also very common in the general population, with an average frequency of 72% in Finland, 50% in Iceland and Sweden and 50% in unaffected individuals in other European populations.[11,25] The homozygous GG haplotype was found in 29% of individuals in the general Finnish population, 25% in Iceland and Sweden populations, and 27% in healthy controls from German and Italy.[11,24] The high-risk haplotype frequencies in the Finnish general population can partly be explained by the high prevalence of exfoliation in Scandinavia (22% of the population >70 years old in Finland), and by the fact that controls were not age matched and some of them might develop XFS and XFG in their later years.

Distribution of three-locus haplotypes was similar among Finnish and other Caucasian populations with European ancestry: GGT was the most significantly associated with exfoliation phenotype, whereas the TGC and GAC haplotypes were underrepresented in exfoliation cases compared with blood donor controls.[11,23–25] The GGT haplotype was present in 93% of the exfoliation cases and 69% of the population-based controls. The GGT increased the risk for XFS and XFG almost 15-fold and the TGC increased the risk nearly fivefold relative to the low-risk GAC haplotype. The estimated PAR value for the GGT haplotype was 52%, whereas PAR for the aforementioned two-loci haplotype GG was 60%.

The *LOXL1* allele and haplotype distribution in XFS and XFG patients versus that in controls was similar in the Finnish case–control and family materials. Allele G of exonic SNP rs1048661 and allele T of intronic SNP rs2165241 were significantly associated with XFS and XFG, when association was measured using independent cases and controls picked from the family. Interestingly, association was not observed for allele G of rs3825942, which showed the strongest association in the case–control material. This is probably because of the small analyzed material and enrichment of G allele both in exfoliation cases and in unaffected relatives in the family. The mean age of the unaffected relatives (n=92) was 62.7, suggesting that a significant number of them might develop XFS in their later years. However, the three-loci haplotype GGT showed strong association with exfoliation in the family and was slightly more common in the family material than in the case–control material. Linkage was not observed for *LOXL1* risk alleles in two- and multipoint analysis. This might be explained by the small family material and low power of linkage methods used to map common variants with low genotypic relative risk.[37]

The *LOXL1* risk-allele frequencies were not significantly different between POAG cases and population-based controls in our study material. This is in agreement with the earlier studies of *LOXL1*

variants in POAG patients.[26,38,39] On the basis of the current data, POAG is a genuinely multifactorial disease, and so far at least 20 genetic loci for POAG have been reported.[40]

In contrast to the Caucasians, *LOXL1* allele and haplotype distribution is different in the Japanese population.[31] The risk-associated allele in Japan at the locus rs1048661 is T instead of the G, and at the locus rs2165241 is C instead of the T in Caucasians. Hence, only the risk allele G of rs3825942 is associated with XFS in both the populations.[31–33] Consistently, the high-risk haplotype in the Japanese is TG, composed of coding SNPs, and TGC, composed of all three SNPs, compared with GG and GGT in Caucasians.[31–35] In the Finnish case–control material, the haplotype GGT increased the risk of XFS and XFG more than threefold, whereas in the Japanese population GGT haplotype was very rare and TGC increased the risk of XFS nearly threefold.[32] The fact that allele G of rs3825942 is the only exfoliation-associated allele in all the populations[31–33] suggests that it is the most likely source of association in the *LOXL1* gene. Moreover, in a study of Caucasian and African-American patients in the United States, a strong association was found for SNPs rs3825942 and rs2165241, but not for rs1048661, and the linkage disequilibrium (LD) between two coding SNPs was very weak ($D'=0.09$, $r^2=0$), which suggests that the effect of rs3825942 is independent of rs1048661.[26] The difference in the alleles flanking the causal G allele of rs3825942 might be explained by the independent origin of the rs3825942 in different genetic backgrounds or by different recombinations around this possibly ancient mutation in the Caucasian and Japanese populations. On the other hand, a strong LD between rs1048661 and rs3825942 was shown in the Nordic ($D'=1$) and Japanese populations ($D'=1$),[11,32,33] and conditional analysis in the Japanese study showed that rs3825942 was highly correlated with rs1048661, which, on the contrary, indicates that rs3825942 is not independent of rs1048661.[32] Hence, the possibility that causative variant is in a state of LD with *LOXL1* SNPs, and yet to be discovered, cannot be excluded.

The disease-associated variants (R141L and G153D) of *LOXL1* are the common ancestral wild-type alleles and are well conserved across mammalian species.[30] This evidence, together with the high prevalence of risk alleles in the general population and the association of risk alleles with XFS and XFG in diverse ethnic groups, suggests that XFS is a normal phenomenon that is associated with aging. However, some elderly people with *LOXL1* risk alleles do not have XFS. The most highly XFS- and XFG-associated haplotypes GG and GGT were present in 42 and 35%, respectively, of the examined over-80-year-old unaffected elderly ($n=26$, mean age 86 years) in our study material. This shows that the age of XFS onset may be very late and vary a lot among individuals. So far it is not known what the protective mechanism delaying the onset of XFS is. Therefore, studies to understand the pathogenesis of XFS should also be directed at identifying factors that prevent the formation of elastin polymer fibers and elastin deposition.

The Australian population had a ninefold lower lifetime incidence of XFS than the Icelandic and Swedish populations, but still had a similar allelic architecture at *LOXL1*.[30] The most highly XFS-and XFG-associated haplotypes were common in the Finnish population-based controls (69–72%) and elderly unaffected controls (35–42%), and one XFS patient homozygous for low-risk haplotype GAC was identified. There is a high sensitivity (95–100%) but poor specificity (3–13%) of *LOXL1* risk alleles as disease predictors.[28] Taken together, the evidence suggests that other genetic (and environmental) factors may influence the development of exfoliation phenotype in addition to *LOXL1*. Several genetic loci have been proposed to be associated with XFS in earlier studies.[12,41] These loci might contain genes that act as protective factors in XFS, which could explain the existence of *LOXL1* risk haplotypes in unaffected elderly individuals. In the original genome-wide scan of our XFS family, the most promising locus for XFS was assigned to 18q12.1–21.33.[12] The role of probable exfoliation-associated variants in chromosome 18 and their possible interaction with *LOXL1* variants warrant further studies.

In conclusion, we have confirmed that there are significant associations between SNPs within the *LOXL1* gene and XFS and XFG also in the Finnish population. Further studies are needed to elucidate the functional mechanism of these SNPs in the accumulation of exfoliation material in the eye.

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