Lack of Association between *LOXL1* Variants and Primary Open-Angle Glaucoma in Three Different Populations

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Purpose. Significant association has recently been reported between pseudoexfoliation glaucoma (XFG) and two single-nucleotide polymorphisms (SNPs), rs3825942, and rs1048661, in the lysyl oxidase-like 1 gene (*LOXL1*). The purpose of this study was to investigate whether XFG-associated variants of *LOXL1* play a significant role in primary open-angle glaucoma in the Caucasian, African-American, and Ghanaian (West-African) populations.

Methods. POAG was defined as the presence of glaucomatous optic nerve damage, associated visual field loss, and elevated intraocular pressure (>22 mm Hg in both eyes). Thirteen tagging SNPs were genotyped by allelic discrimination assays in the Caucasian (279 cases and 227 controls), African-American (193 cases and 97 controls), and Ghanaian (170 cases and 138 controls) populations. Allele and genotype frequencies were compared between the cases and controls from each population.

Results. None of the SNPs associated with XFG in *LOXL1* were significantly associated with POAG in these populations. The risk allele frequencies for rs2165241 and rs3825942 were significantly lower in the African-American and Ghanaian populations, compared with Caucasian individuals.

Conclusions. There was no association between SNPs in the *LOXL1* gene and POAG. This is the first analysis of the *LOXL1* gene in African-American and West-African populations. *LOXL1* gene variants do not appear to play a significant role in the pathogenesis of POAG in populations of either Caucasian or West-African ancestry. (*Invest Ophthalmol Vis Sci.* 2008;49: 3465–3468) DOI:10.1167/iovs.08-1850

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Primary open-angle glaucoma (POAG, OMIM 137760; Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD) is a leading cause of irreversible visual impairment and blindness worldwide, especially in the elderly and the African-American population.[1,2] Many risk factors are associated with the development of POAG, including elevated intraocular pressure, positive family history of glaucoma, refractive error, and African-American race.[1–4] POAG is a complex genetic disorder. Three genes—myocilin, optineurin, and WD repeat domain 36—have been implicated in POAG to date, but together account for less than 10% of POAG cases.[5–7] In addition, a role for variants of the *CYP1B1* gene in POAG has been reported in the Spanish, French, and Indian populations.[8]

Although primary open-angle glaucoma is the most common form of glaucoma in the world, pseudoexfoliation glaucoma (XFG; OMIM 177650) is the most common secondary form of open-angle glaucoma associated with pseudoexfoliation syndrome (XFS; OMIM 177650).[1] XFS is a systemic disorder in which an unidentified fibrillar substance is produced in ocular and many nonocular tissues.[9,10] Its incidence increases with age, and the risk of development XFG in individuals with XFS has been reported to be as high as 60%.[9,10] The prevalence appears to be highest among individuals of Scandinavian ancestry and lower among African Americans.[11] The reported prevalence of XFG varies among ethnic groups and ranges from 0.4% to 28% of cases of open-angle glaucoma in the United States.[11] Recently, variants in the lysyl oxidase like 1 gene (*LOXL1*) were found to be significantly associated with XFG and XFS in a genome-wide genetic association study.[12] Two nonsynonymous coding SNPs in exon 1 of *LOXL1*, rs1048661 (R141L) and rs3825942 (G153D), were found to confer increased risk of XFG in this and other studied populations.[11–16] In the original study, *LOXL1* showed marginally significant association with POAG ($P = 0.04$) in the Icelandic population, but absence of association ($P = 0.18$) in the Swedish population, suggesting that the relationship between POAG and *LOXL1* should be examined in additional populations.[12]

Both POAG and XFG are complex disorders that share a similar clinical phenotype, glaucomatous optic neuropathy. Similar to POAG, XFG is primarily an open-angle form of glaucoma frequently associated with elevated IOP. Since genetic variants associated with *LOXL1* are common in individuals of European ancestry, our goal was to examine whether these variants play a role in POAG in other populations. In this study, we investigated the association between variants of *LOXL1* and POAG in the Caucasian, African-American, and Ghanaian (West-African) populations.

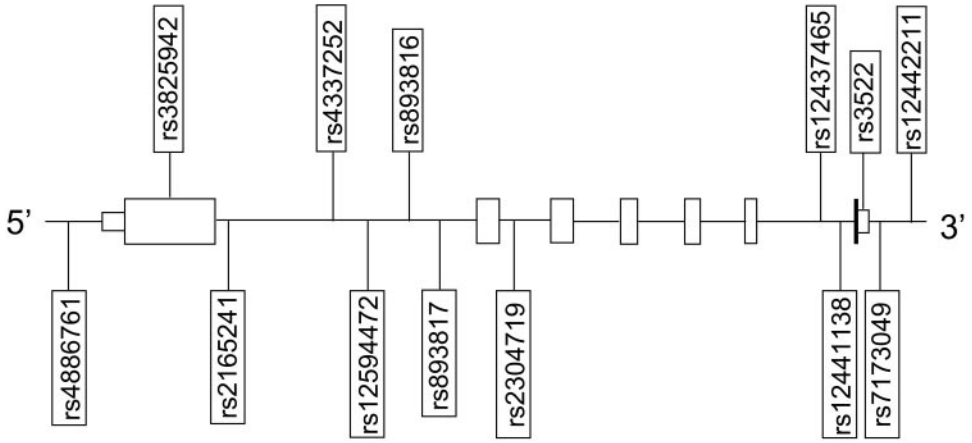

FIGURE 1. Genotyped SNPs in the *LOXL1* gene. *Boxes*: exons; *straight lines*: introns.

METHODS

Subjects

This study adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from all participating individuals. The research was reviewed and approved by the Institutional Review Board from all participating institutions, including Duke University Medical Center (Durham, NC), the Massachusetts Eye and Ear Infirmary (Boston, MA), and, for Ghanaian subjects, Noguchi Memorial Institute of Medical Research of the College of Health Sciences, University of Ghana.

Subjects with POAG were unrelated and met the following three inclusion criteria: (1) intraocular pressure greater than 22 mm Hg in both eyes without medications or greater than 19 mm Hg with two or more medications; (2) glaucomatous optic neuropathy in both eyes; and (3) visual field loss consistent with optic nerve damage in at least one eye.[13] Glaucomatous optic nerve damage was defined as cup-to-disc ratio higher than 0.7 or focal loss of the nerve fiber layer (notch) associated with a consistent glaucomatous visual field defect. Visual fields were performed by using standard automated perimetry.[2] Exclusion criteria included the presence of any secondary form of glaucoma including exfoliation syndrome or a history of ocular trauma. The criteria for unrelated control subjects were: (1) no first-degree relative with glaucoma; (2) intraocular pressure less than 22 mm Hg; (3) no evidence of glaucomatous optic neuropathy; (4) normal visual field by automated perimetry or frequency-doubling test (FDT).

Genomic DNA Genotyping

Genomic DNA was extracted from peripheral blood by standard techniques (Gentra, Minneapolis, MN). HaploView version 3.32 was used to select the tagging SNPs of *LOXL1* in Caucasian (CEU) and African (YRI) samples, using genotype data from the HapMap project (www.hapmap.org).[14] Thirteen tagging SNPs were selected to cover the linkage disequilibrium (LD) blocks of *LOXL1* in both populations (Fig. 1). Allelic discrimination assays (*Taq*Man; Applied Biosystems, Inc., [ABI], Foster City, CA) were used for genotyping these tagging SNPs by use of assay products according to the standard protocols from the manufacturer (Assays-On-Demand; ABI). For quality-control (QC) purposes, two CEPH (the Centre d'Etude du Polymorphisme Humain) standards were included in each 96-well plate, and samples from six individuals were duplicated across all plates, with the laboratory technicians blinded to their identities. Genotype submission to the analysis database required matching QC genotypes within and across plates and at least 95% genotyping efficiency.

Statistical Analysis

Analysis of Hardy-Weinberg equilibrium (HWE) was performed separately for patients and controls from the three populations with GDA software.[15] Within each population, genotype frequencies of POAG cases and controls were compared by logistic regression with adjustment for age and sex (SAS software; SAS Institute, Inc., Cary, NC). SNP genotypes were coded according to a log-additive model, in which the relative risk for carriers of two variant (minor) alleles, compared with the reference group (homozygous wild-type), was assumed to be the square of the relative risk for carriers of one variant. Analysis of variance (ANOVA) was used to test for an effect of genotypes on the mean age of POAG onset or diagnosis. Power calculations were performed with using previously described methods (QUANTO software, http://www.hydra.usc.edu/GxE/ provided in the public domain by the University of Southern California, Los Angeles, CA), assuming a population prevalence of 10% and a log-additive risk model.[16]

RESULTS

The Caucasian dataset contained 279 POAG cases and 227 controls. The African-American dataset contained 193 POAG cases and 97 controls. The Ghanaian (West-African) dataset included 170 POAG cases and 138 controls. A detailed phenotypic summary has been provided elsewhere.[17] Briefly, the mean age at onset or diagnosis was 58.7 ± 12.8 years for the Caucasian, 55.3 ± 13.3 years for the African-American, and 55.4 ± 13.8 years for the Ghanaian patients. The mean age for controls in each population was greater than 55 years. All but two SNPs (rs12441138 and rs893816) were in HWE in both cases and controls from all three populations ($P > 0.05$). Sequencing results generated in 16 samples from the respective populations selected to represent all three possible genotypes confirmed the results of genotyping (*Taq*Man; ABI) (data not shown).

There were no significant genotype or allele frequency differences at the tagging SNPs of *LOXL1* between POAG cases and controls in any of the three populations (Table 1). Of note, the XFG-associated risk allele frequency at SNP rs2165241 was reduced in POAG cases, relative to controls, in the Caucasian population, but not in either African-American or Ghanaian populations. SNP rs4337252 showed marginal association with POAG in the Caucasian data set ($P = 0.023$), but did not survive a Bonferroni correction for multiple testing ($P > 0.05/14$). The age of diagnosis for POAG in all populations was unrelated to the genotyped SNPs ($P > 0.05$). The population-specific allele frequencies of the two XFG-associated variants, rs3825942 and rs2165241, are summarized in Table 2 and compared with previous studies.

Our Caucasian data set had 81% power at 5% significance level to detect an OR of 1.6 or greater for the XFG-associated SNPs (rs3825942 and rs2165241) and >99% power to detect the effect size (OR 3.6 for rs2165241) reported for XFG. For

TABLE 1. Summary of the Allele Frequencies of the 13 Tagging SNPs in the *LOXL1* Gene in POAG Patients and Control Subjects

		Caucasian			African American			Ghanaian		
Marker	Allele	Controls (n = 227)	POAG (n = 279)	P*	Controls (n = 97)	POAG (n = 193)	P*	Controls (n = 138)	POAG (n = 170)	P*
rs4886761	C	0.623	0.634	0.640	0.891	0.857	0.245	0.924	0.919	0.764
rs3825942†	G	0.844	0.829	0.583	0.599	0.617	0.591	0.570	0.622	0.217
rs2165241†	T	0.486	0.424	0.056	0.204	0.237	0.408	0.193	0.226	0.472
rs4337252†	G	0.507	0.433	0.023	0.417	0.427	0.768	0.420	0.467	0.352
rs12594472	A	0.978	0.989	0.169	0.795	0.803	0.861	0.787	0.774	0.767
rs893816	C	0.664	0.611	0.082	0.839	0.836	0.915	0.855	0.865	0.928
rs893817†	A	0.626	0.586	0.212	0.682	0.708	0.527	0.690	0.690	0.906
rs2304719†	C	0.710	0.690	0.535	0.651	0.671	0.602	0.649	0.674	0.614
rs12437465	C	0.433	0.194	0.081	0.321	0.304	0.694	0.314	0.284	0.630
rs12441138	A	0.036	0.180	0.106	0.109	0.105	0.826	0.167	0.159	0.725
rs3522	C	0.590	0.549	0.229	0.380	0.374	0.989	0.410	0.387	0.491
rs7173049	A	0.777	0.768	0.660	0.750	0.775	0.452	0.739	0.754	0.833
rs12442211	A	0.525	0.550	0.425	0.547	0.524	0.554	0.504	0.457	0.330

The single asterisk (*) indicates the probability from the logistic regression with adjustment for age and sex. The double asterisk (†) denotes significant SNPs identified in the original report,[12] and the alleles listed in the table are risk alleles for XFG and XFS.

the two SNPs with lower allele frequency, rs12594472 and rs12441138, the Caucasian sample had 69% and 87% power, respectively, to detect an OR of 2.5 or greater. For all other SNPs, the data sets from all three populations had >92% power at 5% significance level to detect an OR of 2.5 or greater.

DISCUSSION

We found that XFG-associated variants in the *LOXL1* gene are not associated with an increased risk of POAG in the Caucasian, African-American, and Ghanaian (West-African) populations. Thus, our study provides further support that XFG and POAG are genetically and clinically distinct entities. Although the third SNP, rs1048661, shown to be significantly associated with XFG in the Icelandic population was not genotyped in this study,[12] its genetic contribution was covered by rs3825942, since these two SNPs were reported to be in complete LD (r^2 = 1.0) in three independent populations.[12,19,20] Therefore, we evaluated all relevant tagging SNPs and the known coding variants in the *LOXL1* gene.

The allele frequencies of rs2165241 and rs3825942 in XFG and POAG cases as well as unaffected controls are summarized and compared in Table 2.[12,18–23] The frequency of the rs2165241 T allele, which is significantly increased in XFG patients, was decreased in POAG cases compared with control subjects, and this was observed in both U.S. Caucasian and Swedish populations. However, the difference between POAG cases and controls did not reach statistical significance. The risk allele frequencies for rs2165241 T (frequency, 0.42–0.55) and rs3825942 G (frequency, 0.82–0.88) were similar in the Icelandic, Swedish, Australian, Indian, and all three U.S. Caucasian populations, including the present study.[12,18–23] However, the risk allele frequencies were substantially lower in both the African-American and Ghanaian (West-African) populations, with frequencies of 19% for the rs2165241 T allele and 57% for the rs3825942 G allele. The fact that the risk allele

TABLE 2. Summary of the Reported Allele Frequencies of SNPs rs2165241 and rs3825942 in the *LOXL1* Gene Related to Glaucoma

		Allele Frequency			
Population	Affection Status	rs2165241 T*	rs3825942 G†	Sample Size	Reference
Iceland	Control	0.473	0.847	14,474	12
	XFG	0.753	0.987	75	12
	POAG	0.550	0.872	90	12
Sweden	Control	0.535	0.879	198	12
	XFG	0.813	0.995	199	12
	POAG	0.488	0.863	200	12
Japan	Control	NA	0.857	189	18
	XFG	NA	1.0	27	18
India	Control	0.680	0.750	105	19
	POAG	0.675	0.830	112	19
	PACG	0.700	0.755	96	19
U.S. Caucasian	Control	0.486	0.844	227	Liu et al.
	XFG	0.667	0.939	50	20
	POAG	0.424	0.829	279	Liu et al.
African American	Control	0.204	0.599	97	Liu et al.
	POAG	0.237	0.617	193	Liu et al.
Ghana (West Africa)	Control	0.193	0.570	138	Liu et al.
	POAG	0.226	0.622	170	Liu et al.

Both SNPs rs2165241 and rs3825942 were found significantly associated with the increased risk of XFS or XFG in several studies.[12,18,20–22] Based on these studies, the allele T of rs2165241 (*) and the allele G of rs3825942 (†) are considered the risk alleles for XFG.

frequency in the Ghanaian population is as high as 57%, whereas the XFS/XFG prevalence is very low, adds to the body of evidence suggesting that the penetrance of the XFG-associated variant rs3825942 in *LOXL1* is influenced by additional genetic or environmental factors.[24] This supposition is consistent with multiple previous studies supporting the hypothesis that XFS/XFG is a multifactorial and genetically heterogeneous, rather than Mendelian, disease.[25–31]

In conclusion, we have presented the first association analysis of the *LOXL1* gene with POAG in African-American and West-African populations. We found that polymorphisms in the *LOXL1* gene variants do not appear to play a significant role in the pathogenesis of POAG in populations of either Caucasian or West-African ancestry. These data as well as our previous report[20] also clearly indicate that a genetic test for *LOXL1* variants has little if any predictive value for either XFS/XFG or for POAG.

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