Published in final edited form as:

Ophthalmic Genet. 2012 September ; 33(3): 130–133. doi:10.3109/13816810.2011.575430.

Lack of Association between *LOXL1* Gene Polymorphisms and Primary Open Angle Glaucoma in the Saudi Arab Population

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Abstract

Purpose—To investigate whether major single nucleotide polymorphisms (SNPs) in the *LOXL1* gene associated with pseudoexfoliation glaucoma are associated with primary open angle glaucoma (POAG) in the Saudi Arabian population.

Methods—The regions of the *LOXL1* gene associated with pseudoexfoliation glaucoma, encompassing the three common SNPs, (rs1048661, rs3825942 and rs2165241), were sequenced in a Saudi Arabian dataset consisting of 96 POAG cases and 101 healthy controls.

Results—The allele frequency of the G exfoliation risk allele for SNP rs1048661 in POAG cases and controls was 0.75 and 0.76 ($p = 0.886$), respectively and the allele frequency difference was not statistically significant ($p = 0.866$). There was no statistically significant difference in the genotypes between patients and controls ($p = 0.261$ and 0.156 for genotypes G/G and G/T respectively). As for SNP rs3825942, the frequency of the “G” allele in the POAG patients were comparable to that in the controls ($p = 0.477$) and there was no statistically significant difference in genotype G/G and A/G frequency in the study groups. As for SNP rs2165241, the “T” allele frequency in the POAG patients (0.46) was slightly higher than the frequency in controls (0.39), but this difference was not statistically significant ($p = 0.176$).

Conclusion—The Saudi Arabian POAG population, similar to all other populations studied to date, demonstrates no association with SNPs associated with pseudoexfoliation glaucoma.

Keywords

Primary open angle glaucoma; *LOXL1*; Saudi Arabs

Introduction

Glaucoma is a leading cause of visual impairment and blindness throughout the world. Primary open-angle glaucoma (POAG; MIM 137760) is the most prevalent form of

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glaucoma globally. Major risk factors for POAG are elevated IOP, African ancestry, older age, and positive family history.

The prevalence of POAG in Saudi Arabia is unknown. The Glaucoma Unit at King Abdulaziz University Hospital, where approximately 600 new glaucoma patients are seen annually (as indicated by an ongoing retrospective study on the pattern of glaucoma at King Abdulaziz University Hospital for the period from 2006-2010), has found that 19% of those are POAG, 40% primary angle-closure glaucoma, 10% have pseudoexfoliation glaucoma and the remaining 31% are other types of glaucoma.

More than 20 genetic genes or loci have been reported for POAG (1). Two genes, myocilin (*MYOC*) and optineurin (*OPTN*) been identified for POAG. However, disease-associated variants are found in no more than 10% of POAG cases (1-3). The main approaches used to identify the genes associated with glaucoma to date have been the candidate gene approach, genetic linkage analysis, focused association analysis, and genome-wide association analysis. Over 20 genes have been reported to be associated with POAG although in most cases confirmation studies are lacking (4). Additionally, spectrums of mitochondrial abnormalities have been identified in patients with POAG (5).

Thorleifsson and colleagues (6) have reported a genome-wide association study that identified a strong association between three single nucleotide polymorphisms (SNPs) in the lysyl oxidase-like 1 (*LOXLI*) gene. They identified one intronic SNP (rs2165241) and two non-synonymous coding SNPs (rs1048661 and rs3825942) with significant disease association in Icelandic and Swedish subjects. The *LOXLI* gene belongs to the “LOX” family of extracellular enzymes that have multiple functions including cross-linking collagen and elastin through the process of oxidatively deaminating lysine residues. XFS deposits are associated with extracellular matrix and basement membrane proteins, for this reason LOX genes have been viewed as legitimate functional candidates in the pathogenesis of exfoliation (7).

LOXLI SNPs in POAG have been studied in many populations, including American (8), Icelandic (6), Swedish (6), Finnish (9), African American (10), Ghanaian (10), Indian (11), Japanese (12-14), Chinese (15) and South African (16). These studies have uniformly found no association between variants of *LOXLI* and POAG.

This study was conducted to examine whether there is any association between the 3 major *LOXLI* risk alleles for exfoliation glaucoma and POAG in the Saudi Arabian population.

Methods

Study population

The study adheres to the tenets of the Declaration of Helsinki, and all participants signed an informed consent. The study was approved by College of Medicine Ethical Committee (proposal number # 08-657). All study subjects were self identified as Saudi Arabian ethnicity. Family names were all present in the database of Arab families of Saudi Arabian origin. Additionally, these names indicated that all five major Saudi Arabian provinces were represented in the study population. Among the POAG cases and controls, none were related to each other as determined by family (tribal) names and every effort was made to detect and exclude closely related individuals in the study dataset. This analysis was limited to individuals self identified as indigenous Saudi Arabs.

Subjects with clinically diagnosed POAG and healthy controls were recruited into the study at King Abdulaziz University Hospital in Riyadh, Saudi Arabia. All patients and controls

were examined by glaucoma-trained ophthalmologists (SAA and EAO). Patients were eligible for inclusion in this study if they met standard clinical criteria for POAG: i) appearance of the disc or retinal nerve fiber layer e.g. thinning or notching of disc rim, progressive changes, nerve fiber layer defect; ii) the presence of characteristic abnormalities in visual field (e.g. arcuate scotoma, nasal step, paracentral scotoma, generalized depression) in the absence of other causes or explanation; iii) age greater than 40 years and iv) open anterior chamber angles bilaterally on gonioscopy. Exclusion criteria included evidence of secondary glaucoma, e.g. pigmentary dispersion syndrome, pseudoexfoliation, history of steroid use or ocular trauma. All cases had onset of glaucoma after age 40 (adult-onset POAG).

Entry criteria for control subjects were age >40, normal IOP, open angles on gonioscopy, and normal optic nerves on examination.

DNA Analysis

DNA was extracted from peripheral blood using the Illustra blood genomicPrep Mini Spin Kit from GE Healthcare (Buckinghamshire, UK), and stored at -20°C in aliquots until required. PCR amplifications of the region of the *LOXLI* gene encompassing the three common SNPs were performed utilizing the primers described previously (17). Successfully amplified fragments were sequenced in both directions using the M13 forward and reverse primers and the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster city, CA). Fragments were then run on the 3130xl Genetic Analyzer (Applied Biosystems) according to the manufacturer protocol. All the sequenced fragments were then analyzed utilizing SeqScape software v2.6 (Applied Biosystems). Allele frequencies for SNP rs1048661 and rs3825942 were confirmed by repeating the sequencing in both the forward and reverse directions. The sequence of the primers used, the PCR annealing temperature, and the expected amplicon size were described previously (17). All laboratory work reported in this study was carried out at the ophthalmic genetics laboratory, department of ophthalmology, college of medicine, King Abdul-Aziz University Hospital, Riyadh, Saudi Arabia.

Statistical Analysis

Allele frequencies in study groups were compared using the chi-square test. A two tailed p value $< .05$ was considered statistically significant. An exact test for Hardy-Weinberg equilibrium (HWE) of the observed genotypes was performed. Given the known high consanguinity rate in Saudi Arabia [$>65\%$ in some areas (18, 19)] and the possible absence of random mating in this population, we also calculated allelic association p values that were adjusted for deviation from HWE using the method described in Schaid and Jacobsen (1999) (20). All analyses were performed using SPSS v.10 (SPSS Inc., Chicago, IL, USA) and SAS v.9.1 (SAS Institute Inc, Cary, NC, USA) statistical analysis software. Common polymorphisms of *LOXLI* were subjected to Bonferroni correction for multiple testing.

Results

Ninety six POAG cases and 101 controls were analyzed. Of the 96 patients, there were 63 males and 33 females with a mean age of 63.7 (SD 14.7). Of the 101 controls, there were 64 males and 37 females with a mean age of 69.3 (SD 12.4).

For SNP rs1048661, the “G” allele frequency in POAG patients of 75% was almost similar to the rate observed in controls (76.2%; $p = 0.866$). There was no statistically significant difference in the genotypes between patients and controls ($p = 0.261$ and 0.156 for genotypes G/G and T/G respectively) (Table-1).

As for SNP rs3825942, the “G” allele frequency in POAG patients of 84.4% was slightly higher than the rate observed in controls (81.7%), but this difference was not statistically significant ($p = 0.477$). There was no statistically significant difference in the genotypes between patients and controls ($p = 0.755$ and 0.952 for genotypes G/G and A/G respectively) (Table-1).

The third SNP was rs2165241, the “T” allele frequency in POAG patients of 46.4% was higher than the rate of 39.6% observed in controls and this difference was not statistically significant ($p = 0.176$). There was no difference in genotype frequencies between patients and controls ($p = 0.074$ and 0.308 for genotypes T/T and C/T respectively) (Table-1).

Genotypes at SNPs rs1048661, rs3825942 and rs2165241 were all in HWE for both patients and controls ($p = 0.05$). Since the test for deviation from HWE has limited statistical power for moderate sample sizes, and since non-random mating may exist in this study population (18), we also calculated allelic association p-values that are adjusted for deviation from HWE. The p-values in the presence of HWE were 0.774, 0.477 and 0.175 for rs1048661, rs3825942 and rs2165241 respectively. The p-values in the absence of HWE were 0.776, 0.513 and 0.179 for rs1048661, rs3825942 and rs2165241 respectively. These results indicated that the lack of allelic association was robust to the presence or absence of HWE.

Discussion

This is the first study to evaluate major risk alleles in *LOXLI* in subjects with POAG in the Saudi Arabian population. There was no difference in allele association or genotype frequencies between cases and controls for all the three SNPs tested. Our findings are similar to those reported from other parts of the world (6, 8-11, 13-15, 21).

The “G” exfoliation risk allele frequencies for SNP rs1048661 of 76.2% among controls are comparable to the rate observed in other ethnicities and so is the rate of 75% observed in the POAG cases (Table 2). The “T” allele frequency of 46.4% for SNP rs2165241 observed among Saudi POAG patients was comparable to the rate observed among POAG cases from America, Iceland, Sweden, and Finland; slightly higher than in African American, Ghanaian, and Indian populations; and much higher than the rate observed in Asian populations including Japan and China (Table 2). The same can be said about the “T” allele frequency among controls. Allele frequency for cases and controls for the “G” exfoliation risk allele in SNP rs3825942 were 84.4% and 81.7% in, respectively. These frequencies are comparable to the rates observed in other populations, including Asian populations from Japan and China.

We have recently shown that the “G” alleles for SNPs rs1048661 and rs3825942 are associated with the risk of pseudoexfoliation glaucoma (PEG) in the Saudi Arabian cases (17). The lack of association between various *LOXLI* SNPs and POAG reported here provide a further support for the concept that PEG and POAG are genetically distinct.

We have previously reported that *MYOC* and *OPTN* appear to have played a very small role in POAG in the Saudi Arabian population (5). We now confirm that, similar to other populations, variants of *LOXLI* do not contribute meaningfully to the pathogenesis of POAG in this population.

Acknowledgments

The authors thank the Glaucoma Research Chair at the Dept. of Ophthalmology, College of Medicine, King Saud University for funding this study. We would also like to thank Ms. Samar Shehab for her phlebotomy help.

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Table-1

Distribution of the three major *LOXL1* SNPs in POAG patients and controls

SNP	Genotype frequency (%)			Allele frequency (%)		
	T/T	T/G	G/G	T	G	
rs1048661 (g.5758 G>T)						
POAG (n= 96)	8 (8.4)	32 (33.3)	56 (58.3)	48 (25)	144 (75)	
Controls (n= 101)	4 (4.0)	40 (39.6)	57 (56.4)	48 (23.8)	154 (76.2)	
P-value	reference	0.156	0.261	reference	0.866	
rs3825942 (g.5794 G>A)						
POAG (n= 96)	5 (5.2)	20 (20.8)	71 (74)	30 (15.6)	162 (84.4)	
Controls (n= 101)	6 (6)	25 (24.7)	70 (69.3)	37 (18.3)	165 (81.7)	
P-value	reference	0.952	0.755	reference	0.477	
rs2165241 (g.8414 C>T)						
POAG (n= 96)	32 (33.3)	39 (40.6)	25 (26.1)	103 (53.6)	89 (46.4)	
Controls (n= 101)	33 (32.7)	56 (55.4)	12 (11.9)	122 (60.4)	80 (39.6)	
P-value	reference	0.308	0.074	reference	0.176	

LOXL1 indicates lysyl oxidase-like 1; **POAG** indicates primary open angle glaucoma. Nucleotide are numbered as in GenBank accession number NG_011466 (Genomic). * Numbers in parenthesis represent the genotype frequency and allele frequency. P values were calculated when compared to the controls using one genotype or one allele as a reference (indicated).

Table-2

Summary of previous studies investigating the association between the three common *LOXL1* SNPs and POAG in various populations

Cohorts	rs1048661 G%		rs2165241 T%		rs3825942 G%		Reference
	Case	Control	Case	Control	Case	Control	
American	72.4	71.9	41.2	45.6	77.1	79.5	(8)
Icelandic	71.1	65.1	55.0	47.3	87.2	84.7	(6)
Swedish	63.8	68.2	48.8	53.3	86.3	87.9	(6)
Finnish	69.4	68.3	45.7	46.8	78.7	82.3	(9)
Caucasian	NA	NA	42.4	48.6	82.9	84.4	(10)
African American	NA	NA	23.7	20.4	61.7	59.9	(10)
Ghanaian	NA	NA	22.6	19.3	62.2	57.0	(10)
Indian	61.6	69.5	32.1	32.0	83.0	75.0	(11)
Japanese	39.5	49.3	4.8	5.8	91.1	87.7	(12)
Japanese	47.2	45.0	NA	NA	85.0	85.3	(13)
Japanese	51.3	55.4	3.8	12.4	80.0	80.6	(14)
Southern Chinese	42.0	47.2	8.4	10.2	89.4	87.6	(15)
Northern Chinese	47.9	49.7	8.6	8.4	89.9	86.5	(15)
South African	87	81	NT	NT	60	62	(16)
Saudi Arabian	75	76.2	46.4	39.6	84.4	81.7	This study

NT indicates not tested.