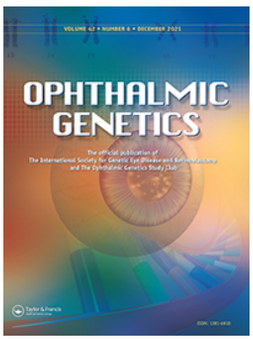

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RESEARCH REPORT

The haplotype of the *CDKN2B-AS1* gene is associated with primary open-angle glaucoma and pseudoexfoliation glaucoma in the Caucasian population of Central Russia

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ABSTRACT

Purpose: To replicate the finding of the association of five *CDKN2B-AS1* gene polymorphisms (rs7865618, rs1063192, rs944800, rs2157719, and rs4977756) with primary open-angle glaucoma (POAG) and to analyze them for possible association with pseudoexfoliation glaucoma (PXFG) in a Caucasian population of Central Russia.

Methods: A total of 932 participants of Russian ethnicity (self-reported), including 328 patients with PXFG, 208 patients with POAG (high-tension glaucoma), and 396 controls, were enrolled in the study. The SNPs were analyzed for possible associations using logistic regression.

Results: Several haplotypes based on the studied SNPs were associated with POAG (three haplotypes) and PXFG (six haplotypes). Haplotype AAAGG of loci rs1063192-rs7865618-rs2157719-rs944800-rs4977756 conferred the highest risk for both POAG (OR = 3.99, p_{perm} = 0.001) and PXFG (OR = 2.84, p_{perm} = 0.001).

Conclusions: The *CDKN2B-AS1* gene was associated with an increased risk of both POAG and PXFG in Caucasians of Central Russia. The gene may be related to the development of various types of glaucoma.

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Association; *CDKN2B-AS1* gene; pseudoexfoliation glaucoma; primary open-angle glaucoma; single nucleotide polymorphism

Introduction

Glaucoma is a group of complex diseases associated with degeneration of the optic nerve and resulting in loss of vision. The number of people suffering from glaucoma is estimated worldwide at about 76.0 million and projected to reach 111.8 million in 2040 (1). Primary open-angle glaucoma (POAG) and pseudoexfoliation glaucoma (PXFG) are among the main causes of permanent blindness worldwide (2). POAG is the most common form of glaucoma and is defined as a progressive optic nerve degeneration resulting in visual field deterioration in eyes with gonioscopically open angles, often with higher intraocular pressure (3). PXFG is a major type of secondary glaucoma, an ocular manifestation of exfoliation syndrome characterized by the deposition of abnormal extracellular fibrillar proteins in the anterior segment of the eye (4). PXFG is more aggressive than POAG, has a poor prognosis and high resistance to therapy (5).

Genetic factors have been suggested as important contributors to the pathogenesis of glaucoma (6,7). Recent genome-wide association studies (GWASs) identified several genetic polymorphisms associated with POAG, including cyclin-dependent kinase inhibitor 2B antisense noncoding RNA (*CDKN2B-AS1*) genomic region on chromosome 9p21.3 (8–14). Additionally, chromosome 9p21.3, where the *CDKN2B-AS1* gene is located, has been identified by GWASs as an important susceptibility locus for optic disc characteristics (optic cup area, vertical cup-disc ratio) (15–18). The cup-disc ratio and optic cup area are the structural features of the optic nerve strongly correlated with glaucoma development (19).

CDKN2B-AS1 controls the transcription of two cyclin-dependent kinase inhibitors 2A and 2B (*CDKN2A* and *CDKN2B*) (20), which affect the proliferation of cells via the TGF-β pathway by arresting the G1-phase of the cell cycle (21). Elevated intraocular pressure (IOP) was reported to induce overexpression of *CDKN2A* and *CDKN2B* in the retina of a rat model of glaucoma (8). Other studies on the animal models also demonstrated that increased IOP is associated with overexpression of *CDKN2B*, which leads to disruption in the cell cycle and causes abnormal cell proliferation (22). Moreover, *CDKN2B* was implicated in the TGF-β signaling pathway (8). The characteristic cupping of the optic nerve head in glaucoma is correlated with the TGF-β expression level as well as with elevated biosynthesis and deposition of extracellular matrix (ECM) proteins (23). Kasetti et al. (24) demonstrated that glucocorticoid dexamethasone activated the TGF-β signaling pathway that increased ECM accumulation, prompted endoplasmic reticulum stress activation in the trabecular meshwork (TM) and significant elevation of IOP. Besides, dexamethasone-induced TGF-β2 in the aqueous humor and TM in the mouse model of ocular hypertension (24). Given that the abnormal ECM accumulation is characteristic of PXFG and may be causally involved in the pathogenesis of both PXFG and POAG (25). This suggests a link between the common physiological risk factor for glaucoma and the

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molecular mechanism that may contribute to the death of the retinal ganglion cells.

This study was aimed to replicate the association of five *CDKN2B-AS1* gene polymorphisms with POAG and to study whether these polymorphisms contribute to the risk of PXFG in a Caucasian population from Central Russia.

Methods

Study subjects

The total size of the study sample was 932 participants, including 328 PXFG patients, 208 POAG (high-tension glaucoma) patients, and 396 controls. The participants were ethnic Russians (self-reported) born in Central Russia (26,27). All participants received ocular examination, including slit-lamp examination, visual acuity, optic disc examination, applanation tonometry, and measurement of central corneal thickness. The criteria for participants with POAG were described elsewhere (28) and included the presence of open anterior chamber angle, high intraocular pressure (≥21 mm Hg), distinctive changes in the optic disc (notching, increased excavation/optic disc ratio, neuroretinal rim thinning), glaucoma-specific visual field defects (narrowing of the field of view with the nose, arcuate or paracentral scotoma), and absence of pseudoexfoliation deposits on the iris, lens capsule, or corneal endothelium of an eye. PXFG was diagnosed based on the following criteria (described elsewhere (29)): the presence of pseudoexfoliation deposits on the iris, lens capsule, or endothelium of the cornea; glaucomatous optic disc changes (thinning and notches of the neuroretinal rim, vertical elongation of the optic cup, larger vertical cup to disc ratio, etc.), visual field defect typical of glaucoma (e.g., nasal step, arcuate defect, paracentral scotoma, temporal wedge) and IOP ≥21 mmHg or controlled IOP on antiglaucomatous treatment in at least one eye. The presence/absence of pseudoexfoliation deposits on the anterior lens capsule was determined by pupillary dilation. The controls had no pseudoexfoliation deposits on the respective structures and no POAG, constant IOP values of <21 mmHg, normal optic discs and no diagnosed pathologies or secondary injuries of the eye at the time of the examination (30).

The participants signed informed consent before enrolment in the study. Their medical examination was conducted at the Department of Eye Microsurgery of St. Joasaph Belgorod Regional Clinical Hospital.

DNA isolation and genotyping assay

About 4–5 ml of blood from each participant was drawn to a tube (Vacutainer®) by a certified nurse. DNA was isolated by the phenol-chloroform method as was described earlier (31,32).

Five SNPs of the *CDKN2B-AS1* gene (rs1063192, rs7865618, rs2157719, rs944800, rs4977756) were selected for the analysis based on the following criteria (33,34): associations with glaucoma and optic disc characteristics reported by GWAS, regulatory potential, MAF > 5%. All selected SNPs had a significant regulatory potential (according to HaploReg, v4.1, https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) (Supplemental Table S1) and were associated with POAG in the previously published GWAS (Supplemental Table S2); three SNPs although were associated with optic disc characteristics by GWAS (Supplemental Table S2).

The polymorphisms were genotyped using the MALDI-TOF mass spectrometry iPLEX platform (Agena Bioscience Inc, San Diego, CA) (45). The quality was checked using blind replicates (42). The repeatability test for 5% randomly selected samples yielded 100% reproducibility.

Statistical analysis

The allele and genotype frequencies were checked for correspondence to the Hardy–Weinberg equilibrium using the chi-square test (46). The association analysis was conducted using the logistic regression method and according to four genetic models, allelic, additive, recessive, and dominant (47). The following variables were used as covariates: age, BMI, systolic and diastolic blood pressure as quantitative variables, whereas essential hypertension, heart ischemia, heart atherosclerosis, family history of glaucoma, and diabetes mellitus (either type I or type II) as qualitative variables (Table 1). The adaptive permutation test (48) was used to correct for multiple comparisons resulting from the analysis of several SNPs, while the Bonferroni correction was applied to adjust for the numbers of the utilized genetic models (n = 4) (40) and the number of groups compared (n = 3). Thus, the cumulative significance level after the Bonferroni correction was set at $p_{perm} \leq 0.004$. Haplotype blocks were identified using the 'confidence intervals' algorithm at D'>0.8 as implemented in HaploView v.4.2 (https://www.broadinstitute.org/haploview/haploview). Haplotype frequencies were determined using the EM-algorithm. Only haplotypes with frequency ≥1% at least in one of the studied groups were included in the analysis. The results of haplotype associations were corrected for multiple comparisons using the approach described above and the significance level at $p_{perm} < 0.017$ (after 1000 permutations and the Bonferroni correction based on the numbers of groups compared, n = 3, i.e., 0.05/3). The association analyses (individual SNPs and their haplotypes) and the permutation test were conducted using the gPLINK v. 2.050 software (PLINK version 1.07) (http://zzz.bwh.harvard.edu/plink/).

Results

The characteristics of the patient (PXFG and POAG) and controls are given in Table 1. The PXFG and POAG patients differed significantly ($p < .001$) from the controls by several parameters: they were older, had elevated systolic and diastolic blood pressure, higher incidence of glaucoma in the family history, higher prevalence of heart atherosclerosis, and heart ischemia. The patients of both cohorts also had a higher prevalence of diabetes ($p < .05$). The PXFG patients had a higher prevalence of essential hypertension ($p < .001$) as compared to the controls. The POAG patients had higher BMI (0.05), higher rates of heart atherosclerosis ($p = .02$), and lower diastolic blood pressure ($p < .001$) compared to the PXFG patients (Table 1). Therefore, all the above factors were used as covariates in the association analyses.

Table 1. Phenotypic characteristics of the study participants.

Parameters	Controls, mean ± SD, % (n)	PXFG patients mean ± SD, % (n)	*p*	POAG patients mean ± SD, % (n)	*p*	P XFG - POAG
N	396	328	–	208	–	–
Age, years (min–max)	62.02±11.54 (42-87)	71.64±8.67 (43-94)	**<0.001**	69.80±8.61 (46-87)	**<0.001**	**0.03**
Gender ratio, m/f	44.44/55.56 (176/220)	47.26/52.74 (155/173)	0.49	43.75/56.25 (91/117)	0.94	0.48
BMI, kg/m2	27.95±5.45	27.57±4.69	0.53	28.42±5.09	0.18	**0.05**
Mean systolic blood pressure, mm Hg	130.87±14.83	142.29±17.67	**<0.001**	139.64±16.01	**<0.001**	0.10
Mean diastolic blood pressure, mm Hg	84.08±9.57	87.24±11.57	**<0.001**	83.89±9.27	0.36	**<0.001**
Smoke	28.03 (111)	25.91 (85)	0.58	26.92 (56)	0.85	0.88
Alcohol consumption	32.07 (127)	30.18 (99)	0.64	30.77 (64)	0.82	0.26
Family history of glaucoma	6.06 (24)	20.12 (66)	**<0.001**	19.23 (40)	**<0.001**	0.88
Ophthalmological characteristics						
Intraocular pressure, mm Hg	16.41±1.54	25.19±5.78	**<0.001**	25.12±5.86	**<0.001**	0.86
Cup to disc ratio	0.25 ± 0.08	0.72 ± 0.31	**<0.001**	0.74 ± 0.35	**<0.001**	0.78
Somatic pathologies						
Essential hypertension	61.11 (242)	72.56 (238)	**0.002**	67.79 (141)	0.13	0.29
Arterial hypotension	5.81 (23)	4.27 (14)	0.44	4.33 (9)	0.56	1.00
Heart atherosclerosis	14.14 (56)	29.57 (97)	**<0.001**	39.90 (83)	**<0.001**	**0.02**
Heart ischemia	24.00 (95)	35.36 (116)	**<0.001**	40.38 (84)	**<0.001**	0.28
Diabetes	10.10 (40)	15.24 (50)	**0.05**	17.31 (36)	**0.02**	0.60
Digestive system pathology	12.88 (51)	14.33 (47)	0.65	14.42 (30)	0.69	1.00
Kidney pathology	7.32 (29)	8.54 (28)	0.64	7.69 (16)	0.98	0.85
Respiratory system pathology	5.05 (20)	6.10 (20)	0.65	6.73 (14)	0.51	0.91
Nervous system pathology	9.09 (36)	9.45 (31)	0.97	10.09 (21)	0.80	0.92

P values <0.05 are shown in bold.

Table 2. Associations of the alleles of the studied SNPs with PXFG and POAG.

SNP	Minor allele	Minor allele frequency cases	Minor allele frequency controls	OR	95% CI L95	95% CI U95	*P*
PXFG							
rs1063192	G	0.415	0.434	0.92	0.75	1.14	0.47
rs7865618	G	0.407	0.431	0.90	0.73	1.12	0.36
rs2157719	G	0.374	0.403	0.88	0.71	1.09	0.25
rs944800	A	0.336	0.354	0.92	0.74	1.15	0.48
rs4977756	G	0.443	0.420	1.10	0.89	1.35	0.38
POAG							
rs1063192	G	0.437	0.434	1.01	0.79	1.29	0.93
rs7865618	G	0.439	0.431	1.03	0.81	1.31	0.80
rs2157719	G	0.413	0.403	1.04	0.82	1.33	0.74
rs944800	A	0.371	0.354	1.08	0.84	1.38	0.56
rs4977756	G	0.468	0.420	1.22	0.96	1.55	0.11

OR, odds ratio;
95% CI, 95% confidence interval.

The data about the analyzed polymorphisms are given in Supplemental Table S3. All SNPs had MAF > 5% and showed no departure from the HWE (p_{bonf}>0.05). None of the SNPs was independently associated with PXFG and POAG according to the allelic, additive, dominant, or recessive models (Tables 2 and 3).

Linkage disequilibrium between the analyzed SNPs was detected in all studied groups: PXFG patients (r^2 = 0.25–0.50, D′ = 0.56–0.77), POAG patients (r^2 = 0.15–0.72, D′ = 0.48–0.86), and controls (r^2 = 0.16–0.75, D′ = 0.46–0.88) (Figure 1). The results of the haplotype association analysis are given in Supplemental Table S4. Several haplotypes manifested significant association with POAG (three haplotypes) and PXFG (six haplotypes) (Table 4). Haplotype AAAGG of loci rs1063192-rs7865618-rs2157719-rs944800-rs4977756 seems to confer the highest risk for both POAG (OR = 3.99, p_{perm} = 0.001) and PXFG (OR = 2.84, p_{perm} = 0.001) (Table 4). The studied groups of the PXFG and POAG patients did not differ by allele and haplotype frequencies.

Discussion

In the present study, we determined the association of five *CDKN2B-AS1* gene polymorphisms (rs1063192, rs7865618, rs2157719, rs944800, and rs4977756) within haplotype blocks with POAG (high-tension glaucoma) and determined their association with PXFG in Caucasians from Central Russia. Haplotype AAAGG of loci rs1063192-rs7865618-rs2157719-rs944800-rs4977756, which was relatively rare in the population (2.76% in controls, 6.62% in PXFG patients, and 8.98% in POAG patients), conferred the highest risk for both POAG (OR = 3.99, p_{perm} = 0.001) and PXFG (OR = 2.84, p_{perm} = 0.001), whereas the most common haplotype AAAGA (differs from the above by just a single variant at SNP rs4977756) demonstrated no association. Besides, in the present study, haplotypes of the *CDKN2B-AS1* gene were associated with high-tension POAG. It should be noted that, while numerous works have reported the association of this gene with normal-tension POAG in European, African Americans, Australians, and Chinese populations (11,35,36,49,50, etc.), some studies showed a relationship between *CDKN2B-AS1* gene polymorphisms and high-tension POAG in different ethnic populations (37,51). Specifically, several SNPs of the *CDKN2B-AS1* gene were associated with high-tension POAG (IOP > 21 mm Hg) in the North Indian Punjabi (51) and Greek (37) populations. Pasquale et al. (19) analyzed a mixed US sample with a predominance (2/3) of POAG patients with IOP ≥ 21 mm Hg and demonstrated the association of the *CDKN2B-AS1* gene not only with the disease, but also with a history of IOP ≥ 21 mm Hg at diagnosis.

The product of the *CDKN2B-AS1* gene is an antisense long non-coding RNA (termed *CDKN2B-AS1* or *ANRIL*). The gene is located in the *CDKN2A-CDKN2B* cluster encoding the p16 (CDKN2A) and p15 (CDKN2B) proteins (44,52). There is ample evidence that all three genes are commonly expressed

Table 3. Associations of the studied SNPs with PXFG and POAG.

SNP	MAF	*n*	Additive model				Dominant model				Recessive model			
			OR	95% CI		*P*	OR	95% CI		*P*	OR	95% CI		*P*
				L95	U95			L95	U95			L95	U95	
PXFG														
rs1063192	G	707	0.79	0.62	1.01	0.064	0.72	0.50	1.05	0.085	0.75	0.48	1.17	0.204
rs7865618	G	716	0.83	0.65	1.60	0.154	0.78	0.54	1.11	0.168	0.81	0.51	1.28	0.371
rs2157719	G	718	0.80	0.62	1.03	0.086	0.75	0.53	1.08	0.121	0.74	0.46	1.21	0.233
rs944800	A	719	0.77	0.60	1.00	0.047	0.74	0.52	1.05	0.089	0.66	0.39	1.12	0.124
rs4977756	G	717	1.01	0.78	1.31	0.929	1.22	0.84	1.77	0.308	0.78	0.49	1.23	0.278
POAG														
rs1063192	G	590	0.89	0.68	1.17	0.404	0.82	0.54	1.24	0.348	0.91	0.56	1.48	0.700
rs7865618	G	598	1.10	0.77	1.32	0.941	0.90	0.60	1.35	0.601	1.20	0.75	1.93	0.453
rs2157719	G	595	0.95	0.73	1.25	0.736	0.75	0.50	1.13	0.169	1.32	0.80	2.16	0.278
rs944800	A	601	0.92	0.69	1.21	0.541	0.91	0.62	1.35	0.639	0.85	0.49	1.50	0.582
rs4977756	G	597	1.15	0.88	1.51	0.314	1.14	0.75	1.73	0.544	1.30	0.81	2.09	0.282

All results were obtained after adjustment for covariates;
OR, odds ratio;
95% CI, 95% confidence interval.

in the ocular tissues related to the pathogenesis of glaucoma, such as the TM, retina, ciliary body epithelial cells, and optic nerve (8,53).

CDKN2B-AS1 is a pleiotropic gene documented for its potential role in cancer, atherosclerosis, coronary artery disease, type-2 diabetes (41,43,54), and regulatory effect on CDKN2A and CDKN2B (55). In patients with coronary artery disease, overexpression of *CDKN2B-AS1* significantly decreased the expression of tumor suppressors *CDKN2A* and *CDKN2B*, thus indicating a reciprocal cis-acting regulatory relationship between these three genes (44). Importantly, heart atherosclerosis, heart ischemia, and diabetes are significant factors ($p < .001$) for the risk of both POAG and PXFG in our study sample. *CDKN2B-AS1* may be an important element of the TGFβ1 signaling pathway by blocking the TGFβ1-induced cell cycle inhibition and contributing to the lower expression level of *CDKN2B* (56). Downregulation of *CDKN2B-AS1* was shown to increase cellular senescence, TGFβ signaling, and ECM deposition in TM cells (57). This suggests that neuroinflammation likely contributes to senescence and death of retinal ganglion cells or glia during the pathogenesis of POAG and PXFG. The recent comprehensive bioinformatics analysis of candidate genes for POAG (termed "POAGome") suggested a key role of the *CDKN2B-AS1, CDKN2B*, and *CDKN2A* genes in the POAG-associated network "Free Radical Scavenging, Glomerular Injury, Organismal Injury and Abnormalities" (55).

Our results about the possible contribution of allele A of loci rs1063192, rs7865618, and rs2157719, and allele G of locus rs944800 (within the multiple haplotypes of the *CDKN2B-AS1* gene) to the development of POAG and PXFG in Caucasians from Central Russia supports the results of the recent GWAS. Specifically, allele A of rs1063192 was associated with a larger vertical cup-disc ratio in Caucasians (38) and was a risk factor for POAG in Japanese (9). Similar results were reported by GWAS for the other abovementioned alleles. The A alleles of rs7865618 and rs2157719 were associated with a higher risk of POAG (10,11,13) and a larger vertical cup-disc ratio and optic cup area (16–18) in different ethnicities. Similarly, allele G of rs944800 is also a risk factor for POAG (14). Our results about the association of the *CDKN2B-AS1* haplotypes with PXFG are in accordance with the previous findings. Specifically, we found that allele A of rs2157719 within haplotype blocks conferred a higher risk for both high-tension POAG and exfoliation glaucoma, which is generally in agreement with the data by Wiggs et al (11) who reported the significant association of the alternative allele G of the locus with both diseases (normal-tension POAG and exfoliation glaucoma).

Despite that several GWAS reported the association of the *CDKN2B* region of 9p21 locus with glaucoma and glaucoma-related endophenotypes (larger vertical cup-disc ratio and optic cup area), thus suggesting it as a risk factor (9,10,11,13,16,17,56,57, etc.), the results of the replicating studies in various populations are often inconclusive and even contradictory (58). While many studies report the association of the *CDKN2B-AS1* gene polymorphisms with glaucoma or its endophenotypes (19,35,38,58–60), the others failed to determine such associations (e.g., 35,51,61). Likewise, we also did

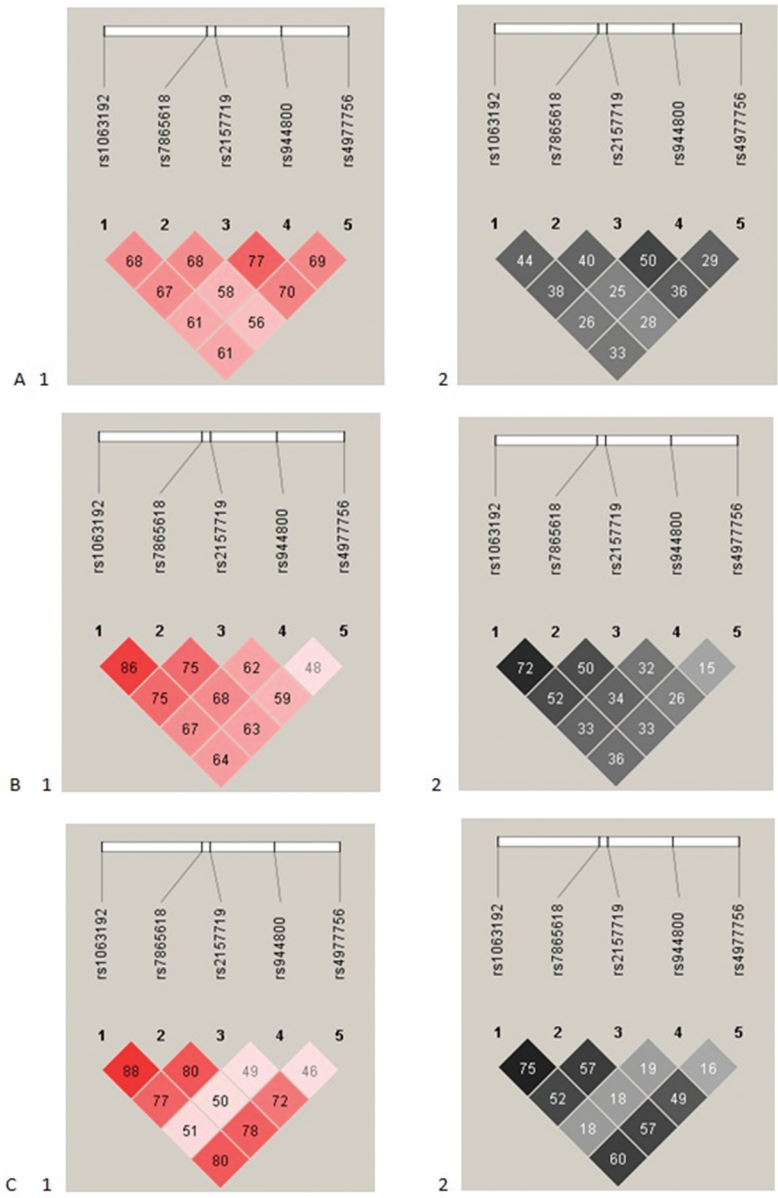

Figure 1. Linkage disequilibrium (LD) between SNPs rs1063192, rs7865618, rs2157719, rs944800 and rs4977756 of the *CDKN2B-AS1* gene in XFG (A), POAG (B) patients, and controls (C). LD values are presented as Lewontin's standardized coefficient D' (left) and the square of the correlation Pearson's coefficient (r^2) (right) between SNPs.

Table 4. Significance associations of the *CDKN2B-AS1* gene haplotypes with PXFG and POAG.

			PXFG patients (n = 328)				POAG patients (n = 208)			
SNPs	Haplotype	Controls (n = 396)	Frequency	OR	$P_{raw\ value}$	P_{perm}	Frequency	OR	$P_{raw\ value}$	P_{perm}
rs1063192-rs7865618	GG	0.3984	0.3347	0.63	0.0007	0.016	0.3935	0.91	0.506	1.00*
rs1063192-rs7865618	AG	0.0260	0.0706	3.00	0.0001	0.004	0.0322	1.82	0.074	0.83*
rs1063192-rs7865618-rs2157719	GGG	0.3431	0.2708	0.60	0.0002	0.008	0.3338	0.86	0.297	0.99*
rs2157719-rs944800-rs4977756	AGG	0.0683	0.1135	2.03	0.0002	0.004	0.1204	2.37	0.0005	0.006
rs7865618-rs2157719-rs944800-rs4977756	AAGG	0.0361	0.0725	3.03	0.0002	0.004	0.0878	2.97	0.0004	0.004
rs1063192-rs7865618-rs2157719-rs944800-rs4977756	AAAGG	0.0276	0.0662	2.84	0.0001	0.001	0.0898	3.99	0.00007	0.001

All results were obtained after adjustment for covariates;
OR, odds ratio;
P, significance level;
*, this indicator is not statistically significant.

not find the individual associations of the analyzed SNPs with the diseases.

The above conflicting findings may be due to various factors. Heterogeneity of the analyzed cohorts and different ethnicity are among the most common (58,62). The other possible causes may include population differences in environmental factors, the prevalence of the multifactorial diseases affecting risk of glaucoma (e.g., type II diabetes mellitus, coronary artery disease, atherosclerosis, etc. (3).) and other potential risk factors, which may or may not be included in the analyses by researchers.

Although we did not find any individual allelic or genotypic associations, certain combinations of alleles within the analyzed haplotypes showed an increased risk for POAG and PXFG in Caucasians from Central Russia. For example, haplotype AAAGG of loci rs1063192-rs7865618-rs2157719-rs944800-rs4977756 conferred the highest risk for both POAG (OR = 3.99) and PXFG (OR = 2.84). The significant association was previously reported by Thakur et al (51) for the CATA haplotype of loci rs3217992-rs1063192-rs2157719-rs4977756, which conferred 1.61-fold risk for primary glaucoma ($p \leq 0.0001$) in the North Indian Punjabi cohort of patients with high-tension POAG (IOP greater than 21 mm Hg), whereas no individual allelic association was found. One of the possible explanations of these results can be a dose-dependent effect, i.e., when several *CDKN2B-AS1* gene alleles with a relatively low individual risk for a disease are present in a genotype, their effects (8,55) are accumulated and become sufficient to pass a threshold necessary for the risk to be detected. In general, information about 9p21 region genotypes may be useful for optimizing glaucoma management when the genetic landscape for glaucoma in different populations becomes ascertained (19).

A comparison of our results with those of Thakur et al. (51) showed that alleles A of the two overlapping loci (rs1063192 and rs2157719) demonstrated the same association in both studies: conferred the highest risk for both high tension POAG (OR = 3.99) and PXFG (OR = 2.84) in Caucasians from Central Russia and high tension POAG in the North Indian Punjabi cohort (OR = 1.61). It is worth noting that the majority of studies reported substitution A→G for rs2157719 (9,11,19, this study, etc.), whereas Thakur et al. (51) and dbSNP (https://www.ncbi.nlm.nih.gov/snp/rs2157719) reported substitution T→C for this locus. This discrepancy is apparently because the different DNA strands of the locus were assumed: according to the complementarity principle, the substitution A→G in one strand corresponds to the T→C substitution in the complementary strand. One overlapping SNP (rs4977756) manifested an opposite effect on glaucoma in Caucasians from Central Russia as compared to the North Indian Punjabi cohort. There may be several factors that contributed to the observed discrepancy at this locus. Among those are: (1) gender bias of the studied samples: predominantly females in our sample and predominantly males in the study (51); (2) different ethnicities of the studied populations: Caucasians from Central Russia vs. North Indian Punjabis, respectively; (3) differences in the utilized methodologies: adjustment for covariates in our study vs. no such adjustment in the study by Thakur et al (51).

The results of the present study suggest that the *CDKN2B-AS1* gene may be one of the syntropic genes for POAG and PXFG. This assumption gains further support from the other studies (e.g. 11). Another gene manifesting the syntropic effect for these diseases is *LOXL1*: its missense variant rs1048661 (R141L) was associated with both PXFG in people of European ancestry (Iceland and Sweden) (63) and POAG in Japanese (14), locus rs2165241 was associated with both PXFG in the North-European populations (63) and POAG in the Mediterranean population (64) and loci rs2165241, rs4886776, rs893818 were associated with both PXFG and POAG in the Russia population (39).

In summary, several haplotypes of five SNPs in the *CDKN2B-AS1* gene were associated with POAG (three haplotypes) and PXFG (six haplotypes) in a Caucasian population of Central Russia. Haplotype AAAGG of loci rs1063192-rs7865618-rs2157719-rs944800-rs4977756 was suggested to confer the highest risk of POAG (OR = 3.99) and PXFG (OR = 2.84).

Disclosure Statement

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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