CDKN2B-AS1 GENE POLYMORPHISM IS ASSOCIATED WITH PRIMARY OPEN-ANGLE GLAUCOMA IN WOMEN OF THE CENTRAL BLACK EARTH REGION, RUSSIA

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Primary open-angle glaucoma (POAG) is a complex disorder. Genetic factors play a vital part in POAG. The prevalence of POAG is gender-specific: the disorder is more often diagnosed in women. Results of the genome-wide association studies (GWAS) strongly support the association of *CDKN2B-AS1* gene polymorphism with POAG. The aim was to perform the replicative study of *CDKN2B-AS1* gene polymorphic loci association with POAG in women of the Central Black Earth Region, Russia. Five *CDKN2B-AS1* gene single nucleotide polymorphisms (SNP), rs1063192, rs7865618, rs2157719, rs944800, and rs4977756, were genotyped in 290 female patients with POAG and 220 female controls. The differences in the haplotype block structure between the POAG patients (no haplotype blocks) and the controls (haplotype block consisting of three SNPs, rs1063192, rs7865618 and rs2157719, was detected) for the set of studied *CDKN2B-AS1* SNPs were revealed using the Solid Spine algorithm (*D'* > 0.8). *CDKN2B-AS1* gene haplotype GGG rs1063192–rs7865618–rs2157719 is associated with POAG in women. This haplotype is considered a protective factor of the disorder (OR = 0.66; p = 0.006, p_{perm} = 0.037).

Keywords: primary open-angle glaucoma, *CDKN2B-AS1*, polymorphism, associations, women

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ПОЛИМОРФИЗМ ГЕНА *CDKN2B-AS1* АССОЦИИРОВАН С ПЕРВИЧНОЙ ОТКРЫТОУГОЛЬНОЙ ГЛАУКОМОЙ У ЖЕНЩИН ЦЕНТРАЛЬНОГО ЧЕРНОЗЕМЬЯ РОССИИ

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Первичная открытоугольная глаукома (ПОУГ) — это многофакторное заболевание, в развитии которого значимую роль играют наследственные факторы. Распространенность ПОУГ имеет гендерные особенности — заболевание чаще выявляют у женщин. Результаты полногеномных исследований (GWAS) свидетельствуют в пользу ассоциации полиморфизма гена *CDKN2B-AS1* с ПОУГ. Целью исследования было репликативное изучение ассоциаций полиморфных локусов гена *CDKN2B-AS1* с ПОУГ у женщин Центрального Черноземья России. У 290 пациенток с ПОУГ и 220 женщин контрольной группы было выполнено генотипирование пяти однонуклеотидных полиморфизмов (SNP) гена *CDKN2B-AS1* — rs1063192, rs7865618, rs2157719, rs944800 и rs4977756. При использовании алгоритма «Solid Spine» (заданный порог *D'* > 0,8) были выявлены различия в структуре блоков сцепления по исследуемым пяти SNP гена *CDKN2B-AS1* между больными ПОУГ (блоки сцепления отсутствовали) и контролем (установлен блок сцепления, состоящий из трех SNP — rs1063192, rs7865618 и rs2157719). У женщин гаплотип GGG rs1063192–rs7865618–rs2157719 гена *CDKN2B-AS1* ассоциирован с ПОУГ — он является протективным фактором развития заболевания (ОШ = 0,66; p = 0,006, p_{perm} = 0,037).

Ключевые слова: первичная открытоугольная глаукома, CDKN2B-AS1, полиморфизм, ассоциации, женщины

Вклад авторов: Н. В. Елисеева — формирование выборки, молекулярно-генетические исследования, подготовка рукописи; И. В. Пономаренко — молекулярно-генетические исследования, статистическая обработка данных, подготовка рукописи; М. И. Чурносов — концепция исследования, редактирование рукописи; все авторы прочли и одобрили окончательный вариант рукописи.

Соблюдение этических стандартов: исследование одобрено этическим комитетом медицинского института Белгородского государственного национального исследовательского университета (протокол № 4 от 19 мая 2015 г.); все участники подписали добровольное информированное согласие на участие в исследовании.

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Glaucoma is a disorder characterized by chronic, progressive optic neuropathy associated with changes in optic nerve head and retinal nerve fiber morphology, which are not attributed to other eye disorders or congenital malformations [1]. Primary open angle glaucoma (POAG) is one the most common forms of glaucoma [2]. The data on the stable sustained incidence rate growth, chronic disease with progressive vision impairment, resulting ultimately in the loss of working ability and disability, demonstrate the sociomedical significance of glaucoma [1]. It should be noted that in the vast majority of cases POAG is diagnosed in patients aged 60–69, commonly having systemic comorbidities. The disorder is almost 1.5 times more often diagnosed in women [1–3].

Genetic factors play a significant role in POAG [4]. Molecular genetic data received to date suggest the involvement of a number of candidate gene polymorphisms in POAG [4–6]. Several genome-wide studies (GWAS) of POAG revealed associations of *CDKN2B-AS1* polymorphic loci with the disorder [7–12].

CDKN2B-AS1 gene is located within the chromosome 9p21 *CDKN2B*– *CDKN2A* gene cluster. *CDKN2B-AS1* belongs to the group of genes responsible for regulation of the long non-coding RNA (lncRNA) synthesis [13]. lncRNA encoded by the gene interacts with polycomb repressive complex-1 (PRC1) and -2 (PRC2), which results in significant epigenetic alterations (histone methylation and monoubiquitination, etc.). This, in

turn, results in significant structural changes of chromatin and directly affects the expression of genes [13]. It should be noted that GWAS data on POAG require replicative studies in various populations, such as Russian population, which have not been subject to replicative studies to date.

The study was aimed to assess the association of the *CDKN2B-AS1* gene single nucleotide polymorphism (SNP) with POAG in women of the Central Black Earth Region, Russia.

METHODS

The sample included 290 female patients with POAG and 220 female controls. Inclusion criteria: Russian ethnicity, place of birth and residence — Central Black Earth Region of Russia [14]. Exclusion criteria: non-Russian ethnicity, place of birth and/or residence — outside the Central Black Earth Region of Russia.

The group of patients included individuals diagnosed with POAG, their diagnosis was verified based on the clinical and instrumental examination data. POAG was diagnosed based on the following criteria [6]: elevated intraocular pressure (IOP over 21 when measured by pneumotonometry, and over 25 when measured by Maklakov tonometry), glaucomatous optic nerve excavation, characteristic changes in the peripheral visual field. The control group included individuals having no POAG (IOP below 21 when measured by pneumotonometry, and below 25 when measured by Maklakov tonometry, no glaucomatous optic nerve excavation and characteristic changes in the peripheral visual field), other eye disorder or severe somatic comorbid condition associated with ocular lesion.

The group of POAG patients and the control group were comparable in terms of age, body mass index (BMI) and somatic comorbidity rate ($p > 0.05$) (Table 1). Ophthalmic examination was carried out in the specialized department of the St. Ioasaph Belgorod Regional Clinical Hospital.

Genomic DNA obtained from peripheral venous blood with phenol–chloroform extraction was subject to genetic analysis [15]. *CDKN2B-AS1* gene single nucleotide polymorphism was selected for analysis based on the following criteria [16]: 1) association with POAG according to previous genome-wide studies; 2) significant regulatory potential; 3) minor allele frequency of 5% or greater.

SNPs were selected for analysis using the catalog of human genome-wide association studies (GWAS Catalog) [17] and the HaploReg database [18]. Five *CDKN2B-AS1* gene SNPs were included in the study: rs1063192, rs7865618, rs2157719, rs944800, and rs4977756. All five SNPs were associated with POAG based on the previous GWAS data [7–12], had a significant regulatory potential (rs7865618, rs2157719, rs944800 are located within the region of histone modifications defined as enhancer marks; rs1063192, rs2157719, rs944800, rs4977756 are located within the DNase I hypersensitive sites; rs1063192, rs2157719, rs944800 are located within the region of various transcription factors regulatory DNA elements), and their minor allele frequency exceeded 5%.

DNA samples were genotyped with the CFX96 Real-Time PCR detection system (Bio-Rad; USA) using the TaqMan probes and the tailor-made kits (TestGene; Russia).

Associations of polymorphic loci with POAG were assessed using logistic regression analysis within the framework of allele (for rs1063192, rs7865618, rs2157719, and rs4977756 polymorphisms alleles G vs. A with a minor allele G were analyzed, and for rs944800 locus it was A vs. G with a minor allele A), dominant (for rs1063192, rs7865618, rs2157719, rs4977756 polymorphisms G/G + A/G vs. A/A were analyzed; for rs944800 A/A + G/A vs. G/G were analyzed), additive (G/G vs. A/G (G/A) vs. A/A) and recessive (for rs1063192, rs7865618, rs2157719, rs4977756 polymorphisms G/G vs. A/G + A/A were analyzed; for rs944800 A/A vs. G/A + G/G were analyzed) genetic models using the plink 1.06 software [19], the data were adjusted for covariate (age). Associations were evaluated using odds ratio (OR) and 95% confidence interval (95% CI). Permutation testing was applied to adjust the results for multiple comparisons. P_{perm} value < 0.05 was considered statistically significant.

Lewontin's standardized disequilibrium coefficient (D') and Pearson correlation coefficient (r^2) were used for assessment of linkage disequilibrium and haplotype block identification between five *CDKN2B-AS1* gene SNPs. Haplotype blocks were analyzed with the Haploview v.4.2 software [20] using the Solid Spine algorithm with $D' > 0.8$. Visualization of linkage disequilibrium between studied *CDKN2B-AS1* SNPs was performed using the Haploview v. 4.2 software. Haplotype frequencies were estimated using the EM algorithm. Associations of haplotypes with POAG were assessed using logistic regression analysis (plink 1.06 software), the data were adjusted for covariate (age) and for multiple comparisons (permutation testing was applied — 1000 permutations). Evaluation of haplotype association with the disorder was performed using odds ratio (OR). P_{perm} value < 0.05 was considered statistically significant [21].

RESULTS

Population genetic analysis showed that distribution of all five *CDKN2B-AS1* SNP genotypes in POAG patients and controls satisfied the Hardy–Weinberg equilibrium ($p_{HWE} > 0.05$) (Table 2).

Table 1. Biomedical and clinical anamnestic characteristics of the studied groups

Parameters	Patients (n = 290)	Controls (n = 220)	p
Age, years	62.24 ± 11.45	61.78 ± 11.06	0.45
BMI, kg/m^2	28.72 ± 5.19	28.57 ± 5.49	0.76
Somatic comorbidities, % (n)			
Cardiovascular system	80.69 (234)	74.55 (164)	0.12
Endocrine system	20.34 (59)	15.91 (35)	0.24
Gastrointestinal tract	14.14 (41)	12.73 (28)	0.74
Urinary tract	7.58 (22)	7.27 (16)	0.99
Respiratory tract	6.55 (19)	5.45 (12)	0.74
Nervous system	18.28 (53)	17.27 (38)	0.86
Other	3.45 (10)	3.18 (7)	1

No significant associations of studied polymorphic *CDKN2B-AS1* loci with POAG were revealed in women (Table 3).

Analysis of linkage disequilibrium between five studied *CDKN2B-AS1* gene polymorphisms using the Solid Spine algorithm (*D*' > 0.8) revealed no haplotype blocks in women with POAG. However, a haplotype block comprising three polymorphisms, rs1063192, rs7865618, and rs2157719, was identified in controls (see Figure). Furthermore, information reported in Figure indicates the existence of recombination hotspot between loci rs2157719 and rs944800 with quite low degree of genetic linkage between rs944800 and rs4977756, as well as high degree of linkage between locus rs4977756 and three polymorphisms (rs1063192, rs7865618 and rs2157719), in the control group. Furthermore, while in the control group there is a zone with *D*' value about 0.4 (see above), then among patients the *D*' value for all studied polymorphisms is about 0.6–0.7.

Association of GGG haplotype of the identified *CDKN2B-AS1* haplotype block rs1063192–rs7865618–rs2157719 with POAG in women was defined. The OR value calculated for this haplotype was 0.66 (p = 0.006 and p_{perm} = 0.037), which demonstrated the protective effect of the haplotype against the disorder in women (Table 4). Association of AGA haplotype with POAG (OR = 5.12; p = 0.009) did not reach statistical significance based on the permutation testing results (p_{perm} = 0.06).

DISCUSSION

Comparison of patients with POAG and female controls based on five *CDKN2B-AS1* gene SNPs revealed the differences in linkage disequilibrium between the studied loci (low degree on genetic linkage between distinct loci in the control group with a D' value of about 0.4, and almost "uniform" linkage of all studied loci in the group of patients with a *D*' value about 0.6–0.7), and the related differences in haplotype block structure (when using the Solid Spine algorithm with *D*' > 0.8, no haplotype blocks were identified in POAG patients, however, in female controls, haplotype block comprising three SNPs, rs1063192, rs7865618 and rs2157719, was identified). Association of GGG haplotype of *CDKN2B-AS1* gene rs1063192–rs7865618–rs2157719 with POAG in women (OR = 0.66) together with no significant independent associations of five studied *CDKN2B-AS1* gene SNPs with the disorder were detected.

It is believed that linkage disequilibrium patterns in modern populations are the result of evolution, which reflects both the demographic history of the population (migration, population subdivision, etc.), and the gene-specific factors, related to mutation and recombination rates, selection, etc. [22]. Despite the fact that the use of LD structure for studying the complex human disorders is limited by the population specificity [22], it is believed that the use of haplotypes for association studies instead of distinct SNPs makes it possible to significantly improve the statistical power of the study, especially where the disease susceptibility loci are not analyzed directly, or in case of high degree multilocus linkage disequilibrium [22, 23]. Genetic distance between the studied loci and the "causative" mutation, as well as allele frequency and the "causative" mutation age, has a direct impact on the haplotype testing efficiency [22].

Regardless of the fact that no obvious "causative" mutations for POAG (for example, nonsense mutations or mutations associated with amino acid substitution) have been detected within the chromosomal region comprising the studied *CDKN2B-AS1* gene SNPs to date, a number of papers report high functional significance of polymorphic loci located within the region (effect on the expression of *CDKN2A*, *CDKN2B*, etc.) [8, 12].

Linkage disequilibrium features detected and related features of haplotype block identification between five studied *CDKN2B-AS1* SNPs in the control group may be just a "particular case" of haplotype structure at the "local scale" of

Table 2. Distribution of *CDKN2B-AS1* gene polymorphic loci in POAG patients and female controls

Polimorfism	Rare allele	Frequent allele	Rare allele frequency	Number of studied chromosomes	Genotype distribution, proportion (%) (homozygous for a rare allele/ heterozygous/homozygous for a frequent allele)	Observed heterozygosity	Expected heterozygosity	Significance level for deviations from Hardy–Weinberg equilibrium (p_{HWE})
POAG patients (n = 290)								
rs1063192	G	A	0.423	568	53/134/97 (18.66/47.18/34.16)	0.472	0.488	0.627
rs7865618	G	A	0.417	566	52/132/99 (18.38/46.64/34.98)	0.466	0.486	0.541
rs2157719	G	A	0.385	564	45/127/110 (15.96/45.03/39.01)	0.450	0.473	0.450
rs944800	A	G	0.338	574	32/130/125 (11.15/45.30/43.55)	0.453	0.448	0.896
rs4977756	G	A	0.476	572	61/150/75 (21.33/52.45/26.22)	0.525	0.499	0.409
Control group (n = 220)								
rs1063192	G	A	0.46	424	43/109/60 (20.28/51.42/28.30)	0.514	0.497	0.679
rs7865618	G	A	0.463	436	47/108/63 (21.56/49.54/28.90)	0.495	0.497	1.000
rs2157719	G	A	0.429	438	41/106/72 (18.72/48.40/32.88)	0.484	0.490	0.890
rs944800	A	G	0.368	440	28/106/86 (12.73/48.18/39.09)	0.482	0.465	0.665
rs4977756	G	A	0.459	438	46/109/64 (21.01/49.77/29.22)	0.498	0.497	1.000

these five loci. As the number of studied loci increases, the overall picture of linkage disequilibrium between multiple loci of this particular chromosomal region is amenable to significant changes (recombination hotspots between distinct loci may be detected in the group of patients as well, the regions of more tight linkage may be revealed in the control group, etc.); in general, at a scale of much larger number of studied loci (compared to five SNPs analyzed during our study) the linkage disequilibrium structure in POAG patients and controls would be similar. Thus, the study [24] aimed to assess linkage disequilibrium and haplotype blocks (the authors used Solid Spine algorithm with D'≥0.75) between 12 *MTHFR* gene SNPs in patients with coronary atherosclerosis and controls revealed three haplotype blocks in patients and two haplotype blocks in the control group. Moreover, the tighter linkage in the *MTHFR* gene 5' region was shown in patients compared to controls. Regardless of the listed above distinct "details" the authors judged about the similarity of LD patterns in the group of patients with coronary atherosclerosis and the control group based on the overall picture of linkage disequilibrium between 12 *MTHFR* gene SNPs (there were similar recombination hotspots, similar haplotype block were detected within the *MTHFR* gene 3' region).

It should be noted that our data on the types of association (genetic risk factor or protective factor) for individual alleles comprising the glaucoma haplotype (haplotype GGG of *CDKN2B-AS1* gene rs1063192–rs7865618–rs2157719 is considered a protective factor for the development of POAG in women, OR = 0.66) are consistent with literature data on the issue. According to the genome-wide study [25], the minor allele G rs1063192 is associated with smaller optic nerve vertical cup-to-disc ratio (β = –0.014 mm^2; p = 6 x 10^{-11}) in the European population (the increased optic nerve vertical cup-to-disc ratio is one of the glaucomatous optic neuropathy symptoms [26]); according to GWAS [9] (performed in Japanese population), minor allele G rs1063192 is also a protective factor for POAG (OR = 0.75; p = 5 x 10^{-11}). Low risk of open-angle glaucoma in individuals (European population) having G rs1063192 in their genotype (both homozygous, OR = 0.76, and heterozygous, OR = 0.85) has been also reported in [27]. Association of allele G rs1063192 with smaller optic nerve vertical cup-to-disc ratio and together with protective effect of the allele against POAG in the European population (OR 0.73) have been reported in [26]. Protective effect of allele G rs1063192 against POAG and other types of glaucoma in the European and Asian populations has been also confirmed by meta-analysis [28]. Thus, our data supporting the protective effect of *CDKN2B-AS1* gene allele G rs1063192 (as a part of GGG haplotype of haplotype block rs1063192–rs7865618–rs2157719) against the disorder in women of the Central

Table 3. Association of *CDKN2B-AS1* gene polymorphism with POAG in women

Loci	Alleles, genotypes	Patients, *n* (%)	Controls, *n* (%)	OR (95% CI)	*p*
rs1063192	Sample size	284	212		
	G vs. A (allele model)	240/328 (42.25/57.75)	195/229 (45.99/54.01)	0.86 (0.67–1.11)	0.24
	G/G vs. A/G vs. A/A (additive model)	53/134/97 (18.66/47.18/34.16)	43/109/60 (20.28/51.42/28.30)	0.82 (0.61–1.10)	0.2
	G/G + A/G vs. A/A (dominant model)	187/97 (65.84/34.16)	152/60 (71.70/28.30)	0.69 (0.44–1.08)	0.1
	G/G vs. A/G + A/A (recessive model)	53/231 (18.66/81.34)	43/169 (20.28/79.72)	0.90 (0.54–1.50)	0.68
rs7865618	Sample size	283	218		
	G vs. A (allele model)	236/330 (41.70/58.30)	202/234 (46.33/53.67)	0.83 (0.64–1.07)	0.14
	G/G vs. A/G vs. A/A (sdditive model)	52/132/99 (18.38/46.64/34.98)	47/108/63 (21.56/49.54/28.90)	0.89 (0.67–1.19)	0.43
	G/G + A/G vs. A/A (dominant model)	184/99 (65.02/34.98)	155/63 (71.10/28.90)	0.74 (0.47–1.15)	0.18
	G/G vs. A/G + A/A (recessive model)	52/231 (18.38/81.62)	47/171 (21.56/78.44)	1.05 (0.63–1.75)	0.85
rs2157719	Sample size	282	219		
	G vs. A (allele model)	217/347 (38.48/61.52)	182/256 (42.92/57.08)	0.83 (0.64–1.07)	0.15
	G/G vs. A/G vs. A/A (additive model)	45/127/110 (15.96/45.03/39.01)	41/106/72 (18.72/48.40/32.88)	0.87 (0.65–1.16)	0.34
	G/G + A/G vs. A/A (dominant model)	172/110 (60.99/39.01)	147/72 (67.12/32.88)	0.74 (0.48–1.16)	0.19
	G/G vs. A/G + A/A (recessive model)	45/237 (15.96/84.04)	41/178 (18.72/81.28)	0.97 (0.56–1.66)	0.91
rs944800	Sample size	287	220		
	A vs. G (allele model)	194/380 (33.80/66.20)	162/278 (36.82/63.18)	0.88 (0.67–1.14)	0.32
	A/A vs. G/A vs. G/G (additive model)	32/130/125 (11.15/45.30/43.55)	28/106/86 (12.73/48.18/39.09)	0.80 (0.59–1.08)	0.15
	A/A + G/A vs. G/G (dominant model)	162/125 (56.45/43.55)	134/86 (60.91/39.09)	0.75 (0.49–1.14)	0.18
	A/A vs. G/A + G/G (recessive model)	32/255 (11.15/88.85)	28/192 (12.73/87.27)	0.74 (0.40–1.37)	0.34
rs4977756	Sample size	286	219		
	G vs. A (allele model)	272/300 (47.55/52.45)	201/237 /(45.89/54.11)	1.07 (0.83–1.37)	0.6
	G/G vs. A/G vs. A/A (additive model)	61/150/75 (21.33/52.45/26.22)	46/109/64 (21.01/49.77/29.22)	1.11 (0.82–1.49)	0.5
	G/G + A/G vs. A/A (dominant model)	211/75 (73.78/26.22)	155/64 (70.78/29.22)	1.22 (0.76–1.93)	0.41
	G/G vs. A/G + A/A (recessive model)	61/225 (21.33/78.67)	46/173 (21.01/78.99)	1.07 (0.64–1.76)	0.81

Note: Results were obtained using the logistic regression model; OR — odds ratio, 95% CI — 95% confidence interval (lower and upper bound of 95% CI); *p* — significance level.

Black Earth Region, Russia (OR = 0.66), are consistent with the data of previous studies.

During the previous studies the following data were obtained for *CDKN2B-AS1* gene allele G rs7865618 being a part of the GGG haplotype of haplotype block rs1063192–rs7865618–rs2157719, which, according to our data, is considered a protective factor of POAG in women of the European Russia (OR = 0.66). According to GWAS [8], allele A rs7865618 increases the risk of POAG in Japanese population (OR = 1.56; $p = 2 \times 10^{-9}$); according to genome-wide studies [29, 30], *CDKN2B-AS1* gene allele G rs7865618 is associated with smaller optic nerve vertical cup-to-disc ratio ($\beta = -0.013$; $p = 3 \times 10^{-20}$ for European population) [29] and smaller area of excavation ($\beta = -0.023$; $p = 1 \times 10^{-21}$ in total for European and Asian populations) [30]. Thus, it is worth noting that our data and the existing literature data on the protective effect of *CDKN2B-AS1* gene allele G rs7865618 against POAG and pathogenetically significant signs of POAG (optic nerve vertical cup-to-disc ratio, area of excavation) fit together.

According to literary sources, *CDKN2B-AS1* gene allele G rs2157719 is associated with low risk of POAG in ethnically diverse populations (in Asian population, in Europeans, and African Americans) [11, 12] and smaller optic nerve vertical cup-to-disc ratio ($\beta = -0{,}013$; $p = 4 \times 10^{-35}$ in total for European and Asian populations) [30]. These data are consistent with our results: allele G rs2157719 being a part of GGG haplotype of haplotype block rs1063192–rs7865618–rs2157719 is considered a protective factor of POAG in women of the European Russia (OR = 0.66).

Despite the fact that a number of GWAS have shown significant associations of *CDKN2B* gene SNPs with glaucoma and related endophenotypes (optic nerve vertical cup-to-disc ratio, area of excavation) [7–12, 25–30], the results of replicative studies performed in various populations are often uncertain, and, in a number of cases, inconsistent, as meta-analysis [28] has shown (*CDKN2B-AS1* polymorphism rs1063192 was analyzed). A number of studies have confirmed association of *CDKN2B-AS1* gene loci with glaucoma/endophenotypes related to glaucoma (optic nerve vertical cup-to-disc ratio) [26, 28, 31, 32]; other studies have revealed no associations of individual *CDKN2B-AS1* gene SNPs with the disorder (for example, rs1063192 and rs4977756 are not associated with POAG in the Indian population [33], in African Americans [34], and in the Pakistan population [35]). The ambiguity of the results obtained by studying the *CDKN2B-AS1* gene loci association with glaucoma are clearly demonstrated by the paper issued in 2021 [33] on meta-analysis of several *CDKN2B-AS1* gene SNPs, including rs1063192, rs2157719 and rs4977756, which were used in our study: thus, of 18 association studies included in the meta-analysis (among them six studies of POAG in Caucasians), significant associations of rs1063192 with POAG have been shown only in 10 studies; significant associations of rs2157719 with POAG have been shown in three of five studies subjected to analysis; only four of 12 papers report significant associations of rs4977756 with the disorder. The study [34], which showed significant association with POAG only in one locus of African Americans out of 24 studied loci (the sample included 1150 patients and 999 controls), can be consider another good example of ambiguous data on association of *CDKN2B-AS1* SNPs with glaucoma; none of these 24 SNPs were associated with the disorder in the population of west Africa (the sample included 483 patients and 593 controls). Significant independent associations of five *CDKN2B-AS1* gene SNP's with POAG have not been revealed during our study as well.

Such ambiguity of the results may be due to clinical heterogeneity of the studied samples of patients, as well as to the differences in the ethnic makeup of the studied populations. Other possible explanations for the ambiguity of association study results are as follows: unique external factors (environmental factors, lifestyle, etc.) in the distinct ethno-territorial groups, the prevalence of various complex disorders contributing to glaucoma заболеваний (atherosclerosis, diabetes mellitus, coronary heart disease, etc.) in these groups [1], as well as the range of environmental glaucoma risk factors related to the listed reasons, which is taken or not taken into account by the researchers during their studies.

Regardless of the fact that the distinct "major" effects of the studied *CDKN2B-AS1* gene loci on POAG in women has not been defined, it has been shown that the combination of certain alleles of the three studied *CDKN2B-AS1* gene SNPs (GGG rs1063192–rs7865618–rs2157719) in the haplotype defines susceptibility to POAG in women of the Central Black Earth Region, Russia. The vital role of the *CDKN2B-AS1* gene haplotypes in susceptibility to POAG has been also shown in the Indian population [33]: CATA haplotype rs3217992–rs1063192–rs2157719–rs4977756 increased the risk of the disorder by 1.61 times ($p \leq 0.0001$), however, the Bonferroni adjusted distinct effects of the listed loci were not statistically significant. It can be assumed, in case of several "risk" *CDKN2B-AS1* gene alleles in the genotype their regulatory effects [8, 12] add up and overcome some threshold essential for glaucoma susceptibility formation in the population, which was tested during our study.

Table 4. Association of *CDKN2B-AS1* gene polymorphic loci rs1063192–rs7865618–rs2157719 haplotypes with POAG in women

Haplotype	Haplotype frequency		OR	*p*
	Patients (*n* = 290)	Controls (*n* = 220)		
GGG	0.287	0.377	0.66	0.006
AGG	0.03	0.021	2.22	0.075
GAG	0.026	0.008	2.87	0.127
AAG	0.039	0.023	1.92	0.145
GGA	0.066	0.049	1.43	0.321
AGA	0.031	0.014	5.12	0.009
GAA	0.045	0.033	1.67	0.215
AAA	0.475	0.475	0.9	0.483

Note: Results were obtained using the logistic regression model; OR — odds ratio; *p* — significance level

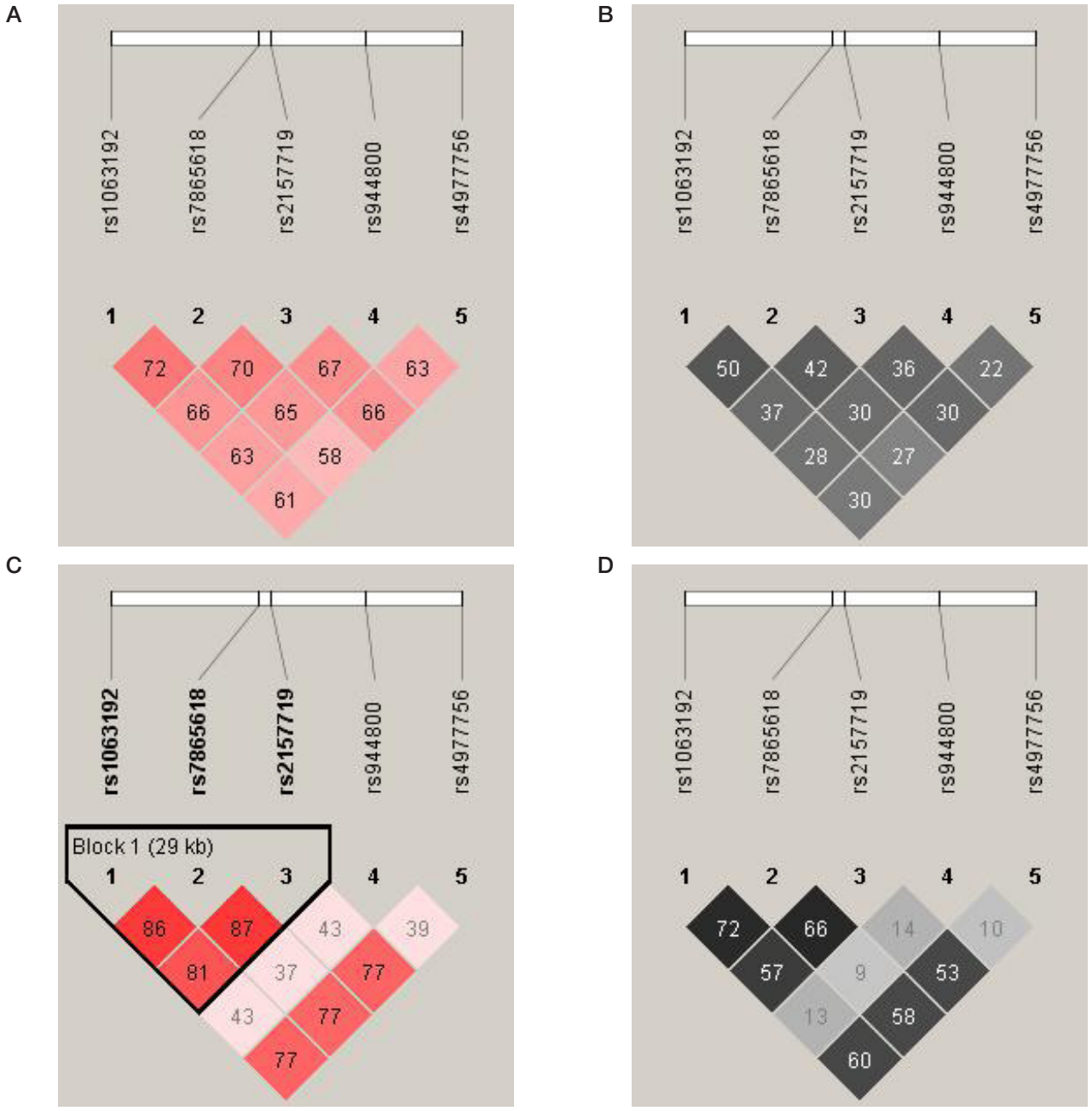

Fig. Linkage disequilibrium patterns between *CDKN2B-AS1* (9p21.3) gene polymorphisms in POAG patients (**A**, **B**) and controls (**C**, **D**) Note: cells in figures on the left contain Lewontin's standardized disequilibrium coefficient *D*'-values (*D*' =1 corresponds to empty cell). Cell color reflects the degree of generic linkage between polymorphisms: *red* — tight linkage (*D*′ = 1; LOD > 2); *pink* — significant linkage (*D*′ < 1; LOD > 2); *white* — weak linkage (*D*′ < 1; LOD < 2). Haplotype blocks are marked by *black* lines. Cells in figures on the right contain Pearson correlation coefficient values (r^2)

CONCLUSION

The data obtained using the Solid Spine algorithm (*D*' > 0.8) indicate the differences in haplotype blocks for five studied *CDKN2B-AS1* gene SNPs between patients with POAG (no haplotype blocks) and controls (haplotype block was identified consisting of three SNPs: rs1063192, rs7865618, and rs2157719). Association of *CDKN2B-AS1* gene GGG haplotype (rs1063192–rs7865618–rs2157719) with POAG in women of the Central Black Earth Region, Russia, has been defined. This haplotype is considered a protective factor for the development of the disorder (OR = 0.66; p = 0.006, p_{perm} = 0.037).

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