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Functionally significant polymorphisms of the *MMP9* gene are associated with primary open-angle glaucoma in the population of Russia

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Abstract

Purpose: The aim of this study was to investigate the role of functionally significant loci of the matrix metalloproteinases genes 1, 3, 9 (*MMP1, MMP3,* and *MMP9*) in the development of primary open-angle glaucoma (POAG) in Caucasians of the Central region of Russia.

Methods: In total 604 participants were recruited for the study, including 208 patients with POAG and 396 healthy controls. They were genotyped at eight single nucleotide polymorphisms (SNPs) of the three *MMP* genes. The association was analyzed using logistic and log-linear regression. POAG-associated loci and their proxies were *in silico* assessed for their functional prediction.

Results: Variant allele G*rs2250889 of *MMP9* was significantly associated with higher risk of POAG ($OR_{cov} = 1.57–1.71$). Haplotype CCA [rs3918242-rs3918249-rs17576] of the *MMP9* gene was associated with lower risk of POAG ($OR_{cov} = 0.33$). Allele A*rs3787268 of *MMP9* was associated with the low intraocular pressure in the POAG patients ($\beta_{cov} = -0.176$ - -0.272), and so were haplotypes AA [rs17576-rs3787268] ($\beta_{cov} = -0.577$) and AAC [rs17576-rs3787268- rs2250889] ($\beta_{cov} = -0.742$) of the same gene, whereas allele 2G*rs1799750 of *MMP1* was associated with the earlier onset of the disease ($\beta_{cov} = -0.112$ - -0.218). *In silico* analysis of the polymorphisms suggested the functionality of POAG-associated SNPs and their proxies (epigenetic potential, expression and alternative splicing effects for several genes).

Conclusions: The *MMP9* gene polymorphisms are associated with POAG and intraocular pressure in POAG patients; rs1799750 of *MMP1* was associated with the earlier age of manifestation of the disease symptoms.

Keywords

Primary open-angle glaucoma, *MMP9*, *MMP1*, SNPs, association

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Introduction

Glaucoma is the most prevalent optic neuropathy determined by multiple genetic and environmental factors and is one of the most common causes of complete blindness.[1] Glaucoma is characterized by progressive deterioration of ganglion cells and optic nerve, gradual decrease of the visual field, and vertical elongation of optic disc cupping. Based on the anterior segment anatomy, glaucoma is classified as primary open-angle glaucoma (POAG) or primary angle-closure glaucoma (PACG).[2] POAG is the most common type of glaucoma that is characterized by specific glaucomatous retinal, optic nerve, and clinical findings without a clear secondary cause.[3] In 2015, 57.5 million people worldwide were

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affected by POAG, including 7.8 million people in Europe alone. The prevalence of the disease was estimated at 2% in Europe and 2.2% globally.[4]

POAG is a progressive optic neuropathy characterized by loss of ganglion cells and deterioration of the visual field in eyes with gonioscopically open angles, either with or without increased intraocular pressure (IOP).[5] Matrix metalloproteinases (MMP) are proteins, which have been implicated in the development of POAG.[6,7] MMPs are zinc and calcium-dependent endopeptidases involved in homeostasis and remodeling of the extracellular matrix (ECM).[8] Importantly, MMPs are important regulators of the aqueous humor outflow from the eye anterior chamber and therefore significantly affect intraocular pressure.[7] Patients with diagnosed POAG have an altered MMPs level in the aqueous humor.[6,7] The extracellular matrix pathway was previously suggested to be involved in optic nerve degeneration in the GWAS (genome-wide association study) of POAG.[9,10]

The *MMP* genes were suggested to play an important role in the development of various glaucoma types, including POAG.[11] Several studies have been conducted to analyze polymorphic variants of the *MMP* for their possible contribution to POAG.[12–21] Several loci of the *MMP* genes (rs3918242, rs3918249, rs17576 *MMP9,* rs1799750 *MMP1,* etc.) were associated with POAG in different populations.[13–17,19–21]

The studies of European populations are limited[12–14,22] and reported only two *MMP* polymorphisms (rs1799750 *MMP1* and rs3918242 *MMP9*) as associated with POAG.[13,14] Surprisingly, despite the small number of gene association studies of the *MMP* genes and glaucoma, the number of their meta-analyses is quite noticeable.[16–18,20] Moreover, different meta-analyses have been conducted on the same two experimental works (e.g..[18,20] Such a practice leads to dubbing results of just a few experimental studies and misleading generalizations. As such, expanding experimental studies on the association of the *MMP* genes with POAG in different populations rather than doing meta-analyses of limited data seems justified. The experimental data can further be comparatively analyzed to determine ethnic and geographic differences of the MMP polymorphisms underlying POAG.

This study was aimed to analyze functionally important loci of the three *MMP* genes (*MMP1, MMP3*, *MMP9*) for their possible role in POAG in Caucasians of Central Russia.

Materials and methods

Study subjects

The protocol of the study was approved by the Regional Ethics Committee at the medical institute of Belgorod State National Research University (protocol #3 of 12 April 2013). All participants were requested to sign informed consent documents before entering the project. In total 604 individuals were enrolled, including 208 patients with POAG and 396 control group subjects. Only subjects born in the central region of Russia and ethnic Russians (self-reported) were qualified for the study. The POAG patients were enrolled according to the criteria described elsewhere[23]: open anterior chamber angle, optic disc parameter changes (neuroretinal rim thinning, notching, increased excavation and ratio of the optic disc), high intraocular pressure (≥21 mm Hg), glaucoma-specific visual field defects (arcuate or paracentral scotoma, narrowing of the field of view with the nose), the lack of secondary glaucoma conditions. We used the following exclusion criteria for control group participants: presence of POAG, PACG, acute eye disorder, or any diseases causing the secondary injury of eyes at the time of the survey (exfoliation glaucoma, etc.).[24] The absence/presence of exfoliation deposits on the anterior lens capsule was determined on all participants by pupillary dilation.[25] All participants (case and control) had no signs of exfoliation glaucoma (exfoliation material on anterior segment structures).

A clinical examination (diagnostics of POAG and somatic pathologies) of all participants (POAG patients and control group subjects) was performed at the St Ioasaf Belgorod Regional Clinical Hospital (Department of Eye Microsurgery).

Deoxyribonucleic acid (DNA) extraction, SNPs selection, and genotyping

Total DNA was isolated from peripheral blood according to the common phenol-chloroform DNA extraction protocol.[26]

Eight loci of the three *MMP* genes: *MMP1* (rs1799750), *MMP3* (rs679620), and *MMP9* (rs3918249, rs17577, rs17576, rs3787268, rs2250889, rs3918242), were chosen. For *MMP* loci selection, the following criteria were applied[27,28]: 1) previously reported associations with glaucoma (POAG, etc.), 2) epigenetic (regulatory) potential, 3) SNP minor allele frequency (MAF) > 0.05.

All selected SNPs were functionally important as evidenced by HaploReg[29] (Table 1); seven polymorphisms were glaucoma-associated (including five POAG-associated loci) according to the previously published studies (Supplementary Table 1). Although rs679620 of the *MMP3* gene was not glaucoma-associated in previous reports, it was associated with several POAG risk factors (essential hypertension, blood pressure, atherosclerosis disease, etc.).[30,31]

The *MMP* loci were genotyped by the MassARRAY 4 system (Agena Bioscience Inc, San Diego, CA). Approximately five percent of the DNA samples were randomly regenotyped[32] and showed complete (100%) reproducibility.

Table 1. The regulatory potential of the studied SNPs.

chr	pos (hg38)	variant	Ref	Alt	AFR freq	AMR freq	ASN freq	EUR freq	GERP cons	SiPhy cons	Promoter histone marks	Enhancer histone marks	DNAse	Proteins bound	Motifs changed	NHGRI/EBI GWAS hits	GRASP QTL hits	Selected eQTL hits	GENCODE genes	RefSeq genes	dbSNP func annot
20	46007337	rs3918242	C	T	0.12	0.09	0.17	0.17				BLD, THYM, SPLN	IPSC		4 altered motifs		1 hit	6 hits	1.6 kb 5' of MMP9	1.6 kb 5' of MMP9	
20	46009497	rs3918249	T	C	0.56	0.31	0.72	0.39			4 tissues	BLD	BLD, BLD		4 altered motifs			9 hits	MMP9	MMP9	intronic
20	46011586	rs17576	A	G	0.35	0.28	0.72	0.39			17 tissues	4 tissues	ESC		Pax-4			12 hits	MMP9	MMP9	missense
20	46013092	rs3787268	G	A	0.09	0.16	0.36	0.21			BLD, SKIN	BLD	BLD, BLD		6 altered motifs			4 hits	MMP9	MMP9	intronic
20	46013767	rs2250889	G	C	0.82	0.80	0.76	0.95			24 tissues		15 tissues	CTCF	NRSF				MMP9	MMP9	missense
20	46014472	rs17577	G	A, C	0.17	0.10	0.18	0.18			21 tissues	SKIN	22 tissues	4 bound proteins			1 hit	6 hits	MMP9	MMP9	missense
11	102842889	rs679620	T	C	0.71	0.64	0.67	0.54							p300			3 hits	MMP3	MMP3	missense
11	102799764	rs1799750	TC	T	0.55	0.44	0.33	0.49				6 tissues	5 tissues	CFOS, GATA2	21 altered motifs			8 hits	1.6 kb 5' of MMP1	LOC100288077	intronic

Table 2. Phenotypic characteristics of the study participants.

Parameters N	Controls, mean ± SD,% (n) 396	POAG patients mean ± SD,% (n) 208	p -
Age, years (min–max)	62.02 ± 11.54 (42-87)	69.80 ± 8.61 (46-87)	**<0.001**
Women	55.56 (220)	56.25 (117)	0.94
Body mass index, kg/m2	27.95 ± 5.45	28.42 ± 5.09	0.18
Mean systolic blood pressure, mm Hg	130.87 ± 14.83	139.64 ± 16.01	**<0.001**
Mean diastolic blood pressure, mm Hg	84.08 ± 9.57	83.89 ± 9.27	0.36
Smoke	28.03 (111)	26.92 (56)	0.85
Alcohol	32.07 (127)	30.77 (64)	0.82
Family history of glaucoma	6.06 (24)	19.23 (40)	**<0.001**
Ophthalmological characteristics			
Intraocular pressure, mm Hg	16.41 ± 1.54	25.12 ± 5.86	**<0.001**
Cup to disc ratio	0.25 ± 0.08	0.74 ± 0.35	**<0.001**
Somatic pathologies			
Essential hypertension	61.11 (242)	67.79 (141)	0.13
Arterial hypotension	5.81 (23)	4.33 (9)	0.56
Heart atherosclerosis	14.14 (56)	39.90 (83)	**<0.001**
Heart ischemia	24.00 (95)	40.38 (84)	**<0.001**
Diabetes	10.10 (40)	17.31 (36)	**0.02**
Digestive system pathology	12.88 (51)	14.42 (30)	0.69
Kidney pathology	7.32 (29)	7.69 (16)	0.98
Respiratory system pathology	5.05 (20)	6.73 (14)	0.51
Nervous system pathology	9.09 (36)	10.09 (21)	0.80

Statistically significant P values are given in bold.

Data analysis

Both case and control groups were examined for correspondence of genotype and allele frequencies to the Hardy-Weinberg equilibrium (HWE) using the common χ^2 test The association between the SNPs and POAG risk was assessed by logistic regression (considering the dominant, recessive, and additive models)[33] and odds ratios (ORs) with 95% confidence intervals (CIs). The regression analysis was adjusted for quantitative (age and systolic blood pressure) and qualitative (the presence of heart atherosclerosis and ischemia, diabetes, and a positive glaucoma family history) covariates (Table 2). Linkage disequilibrium plots were constructed in HaploView[34] (using the 'solid spine' algorithm and parameter D' > 0.80). The association analysis computations were conducted using PLINK v. 1.07[35] with a correction for multiple comparisons (permutation test).[36] The significance level was set at $p_{perm} \leq 0.05$. Statistical power for each SNP was computed using Quanto 1.2.4.[37]

The POAG patients were analyzed for association between the *MMP* loci and several clinical characters of the disease: intraocular pressure, age of the disease manifestation, and systolic blood pressure. Systolic blood pressure was included in the analysis based on the following considerations. First, elevated systolic blood pressure is a risk factor for POAG according to both our (Table 2) and the literature data.[1,5] Second, the *MMPs* analyzed in the present study may contribute to both POAG (see Supplementary Table 1) and cardiovascular disorders associated with the increased systolic blood pressure (e.g. arterial hypertension, stroke on the background of arterial hypertension, and the others).[38–42] Since the values of intraocular pressure, age of the disease manifestation, and systolic blood pressure in the sample were not normally distributed (according to the Shapiro-Wilk test), they were transformed using the QQ-plot function in the R programming environment.[43] Association between a SNP minor allele and the above traits was analyzed using log-linear regression assuming the three principal genetic models (additive, recessive, and dominant) with a correction for the above covariates and adjustment for multiple comparisons by the permutation test.[36] The computations were conducted using PLINK v. 1.07.[35] The significance level was set at $p_{perm} \leq 0.05$.

SNPs functionality effects

To estimate the potential downstream functional effects of the POAG-associated variants and their proxies,[44,45] we used the available data on epigenetic effects (HaploReg,[29]) non-synonymous functional predictions (SIFT[46] and PolyPhen-2[47] databases), expression (eQTL) (Blood eQTL browser[48] and Genotype-Tissue Expression (GTE) × Consortium atlas[49]) and alternative splicing (sQTL) quantitative traits (GTEx Consortium atlas.[49]) HaploReg was used to identify variants in strong linkage

disequilibrium (LD, $r^2 \geq 0.80$)[50] with the POAG-associated variants.

Results

The summary of the phenotypic parameters of the participants (POAG-affected and control groups) is provided in Table 2. A positive glaucoma family history, heart disorders (atherosclerosis and ischemia), and diabetes mellitus were significantly more prevalent in the cases versus the controls. Also, the control subjects were younger and had lower systolic blood pressure as compared to the POAG-affected participants. Therefore, these characteristics were applied as covariates in the regression association analyses.

Supplementary Table 2 shows the data about the studied SNPs. No departure from HWE for all examined loci was detected. Among the analyzed SNPs, only those of *MMP9* showed association with POAG. Specifically, allele G*rs2250889 conferred a higher risk for POAG: the odds ratio adjusted for covariates $OR_{cov} = 1.57$, $p_{perm} = 0.036$, power 70.02% according to the additive model, and $OR_{cov} = 1.71$, $p_{perm} = 0.022$, power 76.70% according to the dominant model (Table 3). Haplotype CCA [rs3918242-rs3918249-rs17576] (Figure 1) was associated with POAG ($OR_{cov} = 0.33$, $p_{perm} = 0.030$) (Table 4).

Locus rs3787268 *MMP9* was associated with the intraocular pressure level and locus rs1799750 *MMP1* - with age of the disease manifestation (Table 5). Specifically, allele A*rs3787268 was associated with the lower intraocular pressure in POAG patients ($\beta_{cov} = -0.176$, $p_{perm} = 0.048$ according to the additive model; $\beta_{cov} = -0.272$, $p_{perm} = 0.005$ according to the dominant model), whereas allele 2G*rs1799750 appeared to be a risk factor for the earlier age of the disease manifestation ($\beta_{cov} = -0.112$, $p_{perm} = 0.007$ for the additive model; $\beta_{cov} = -0.218$, $p_{perm} = 0.0008$ for the dominant model) (Table 5). Besides, haplotypes AA [rs17576-rs3787268] ($\beta_{cov} = -0.577$, $p = 0.003$, $p_{perm} = 0.012$) and AAC [rs17576-rs3787268- rs2250889] ($\beta_{cov} = -0.742$, $p = 0.006$, $p_{perm} = 0.035$) were associated with intraocular pressure.

Functional SNP

Two POAG-associated SNPs were missense. Polymorphism rs2250889 results in an amino acid substitution Arg574Pro (SIFT score = 1.00 and "tolerated" predictive parameter; PolyPhen-2 score = 0.00 and predictive value "benign"); rs17576 is a missense variant Gln279Arg (SIFT score = 0.288, predictive parameter "tolerated"; PolyPhen-2 score = 0.004, predictive value "benign",). Also, the POAG-associated polymorphism rs3918242 is in strong LD with a missense variant rs17577 of the same gene, which causes amino acid change Arg668Gln (SIFT parameter "tolerated", score = 0.647; PolyPhen-2 parameter "benign", score = 0.010).

The analysis by HaploReg indicated that all four POAG risk SNPs possessed a regulatory (epigenetic) potential (Table 1): they are located in evolutionarily conserved regions that have promoter and enhancer histone marks, the Deoxyribonuclease I (DNAase I) hypersensitive regions in various types of cells, tissues, and organs, a site of DNA binding to CTCF regulatory protein, and a genomic region with 12 transcription factors (TF) binding loci. Variant allele G*rs2250889 confers lower affinity to TF NRSF (difference between the log-odds scores (ΔLOD) of the alternative (alt) and reference (ref) alleles equal to −11.4), allele C*rs3918242 increases affinity to four TFs (Ahr, ΔLOD = 11.9; Arnt, ΔLOD = 11.9; HIF1, ΔLOD = 11.9; Myc_disc9, ΔLOD = 11.3), and decreases affinity to E2F (ΔLOD = −4.0) and Myc_known8 (ΔLOD = -1.7), variant allele C*rs3918249 decreases affinity to two TFs (Arid3a and Pax-5, ΔLOD equals to −0.7 and −3.9, respectively) and increases affinity to two TFs (Hmx and Hoxb8, ΔLOD equals to 1.9 and 2.9, respectively) and allele A*rs17576 increases affinity to TF Pax-4 (ΔLOD = 2.1).

We also performed the *in silico* analysis of 38 SNPs, which were linked to the four POAG-associated SNPs (Supplementary Table 3). Among those, ten SNPs were located in an evolutionarily conserved region. Most of the proxy SNPs possessed significant epigenetic effects. For example, rs2236416 (linked to rs3918242) is located in the DNA binding site with modified histone marking promoters (15 tissues) and enhancers (blood cells), in the hypersensitive region to DNAase-I (33 tissues), a binding site of six regulatory proteins, and a region of regulatory motif NF-Y.

Loci rs3787268 *MMP9* and rs1799750 *MMP1* associated with the POAG-related clinical traits also demonstrated significant epigenetic effects (Supplementary Table 3). Specifically, rs1799750 is located in regions that have enhancer histone marks and DNAase I hypersensitive site (in 6 and 5 organs/tissues respectively, a site DNA binding to CFOS and GATA2 regulatory proteins, and a genomic region with 21 TF-binding loci (Supplementary Table 3).

According to the Blood eQTL browser, rs3918242 is linked to seven local eQTL *(cis*-eQTL) loci affecting the messenger ribonucleic acid (mRNA) transcription level of three genes (*AL162458.10-3, DNTTIP1,* and *MMP9*) in peripheral blood ($p_{FDR}<0.05$) (Supplementary Table 4). Expression of *AL162458.10-3* and *MMP9* in peripheral blood may also be affected by two loci linked to rs3787268 *MMP9.* None of the SNPs was identified as the distant eQTL (*trans*-eQTL) one ($p_{FDR} >0.05$).

According to the GTExportal database, all POAG-associated SNPs had eQTL significance ($p_{FDR}\leq0.05$) and were associated with the transcription level of ten genes (*SNX21, PCIF1, CD40, PLTP, ZSWIM1, NEURL2,*

Table 3. Associations of the studied SNPs with POAG.

				Additive model				Dominant model				Recessive model			
					95%CI				95%CI				95%CI		
SNP	Gene	Minor allele	n	OR	L95	U95	P	OR	L95	U95	P	OR	L95	U95	P
rs1799750	*MMP1*	2G	587	0.91	0.70	1.19	0.507	0.85	0.56	1.30	0.461	0.92	0.58	1.47	0.732
rs679620	*MMP3*	T	591	0.97	0.74	1.00	0.803	0.97	0.63	1.48	0.878	0.94	0.60	1.48	0.794
rs3918242	*MMP9*	T	593	1.01	0.70	1.44	0.979	1.11	0.73	1.70	0.620	0.51	0.16	1.62	0.257
rs3918249	*MMP9*	C	601	0.81	0.62	1.06	0.125	0.81	0.55	1.20	0.301	0.66	0.39	1.11	0.116
rs17576	*MMP9*	G	584	0.99	0.75	1.32	0.971	0.94	0.63	1.41	0.764	1.10	0.64	1.88	0.740
rs3787268	*MMP9*	A	599	0.82	0.59	1.14	0.244	0.76	0.51	1.15	0.192	0.88	0.38	2.04	0.763
rs2250889	*MMP9*	G	591	**1.57**	**1.04**	**2.37**	**0.032**	**1.71**	**1.06**	**2.76**	**0.028**	1.72	0.47	6.27	0.411
rs17577	*MMP9*	A	588	1.14	0.81	1.60	0.446	1.16	0.77	1.75	0.467	1.23	0.49	3.07	0.659

All results were obtained after adjustment for covariates (age and systolic blood pressure were applied as quantitative variables (value of the trait), while family history of glaucoma, the presence of heart atherosclerosis, heart ischemia, and diabetes (either type I or type II) were used as qualitative variables (yes / no)). *SNP*, single nucleotide polymorphism, n – the total number of participants (case and control), *OR*, odds ratio, *95%CI*, 95% confidence interval (*L95*, lower limit, *U95*, upper limit), *P*, significance level. Statistically significant values are given in bold.

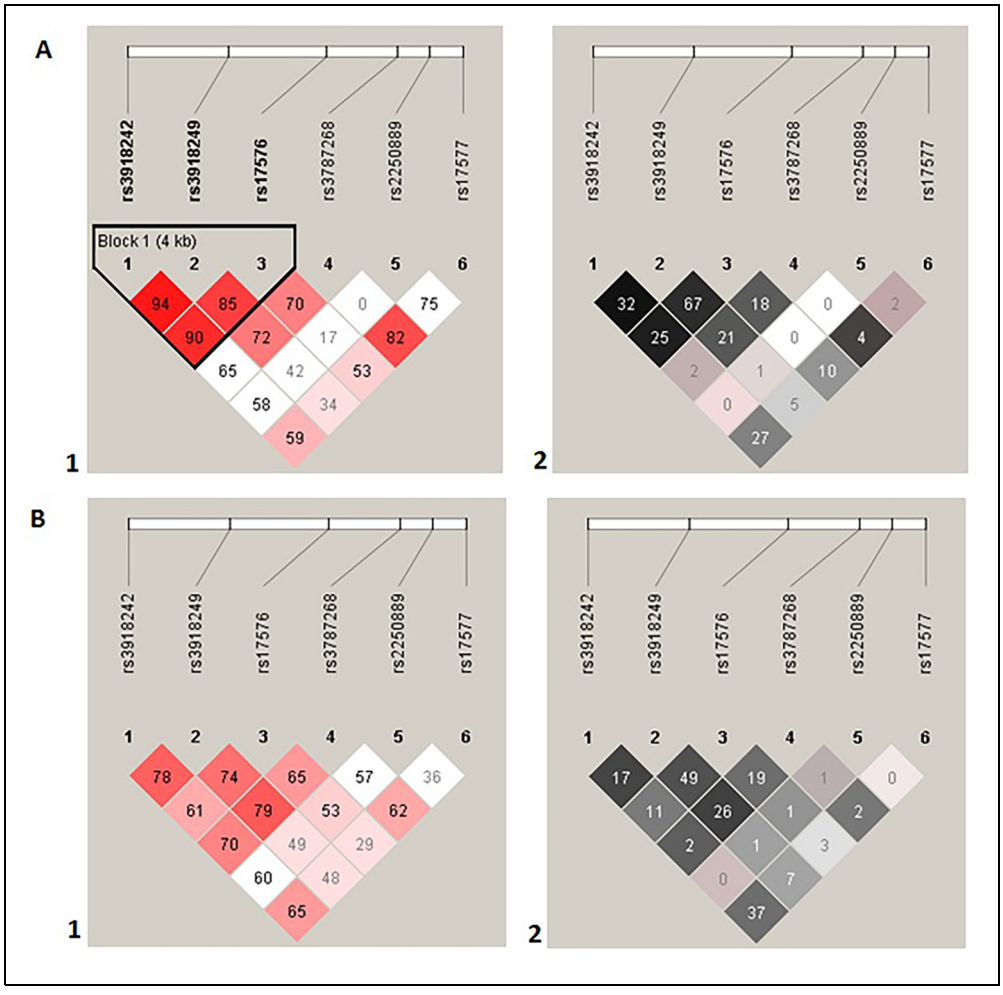

Figure 1. Linkage disequilibrium (LD) between SNPs rs3918242, rs3918249, rs17576, rs3787268, rs2250889, and rs17577 of the *MMP9* gene in POAG patients (A) and control group (B). The LD values are shown as Lewontin's standardized coefficient D′ (sections 1) and the square of the correlation Pearson's coefficient (r^2) (sections 2) between the SNPs.

Table 4. Associations of the rs3918242-rs3918249-rs17576 haplotypes of the *MMP9* gene with POAG.

Haplotype	Controls (n = 396)	POAG patients (n = 208)	OR	P	P_{perm}
TCG	0.1261	0.1514	1.21	0.340	0.898
CCG	0.2103	0.1855	0.87	0.443	0.962
CTG	0.0495	0.0544	0.99	0.970	1.000
TCA	0.0241	0.0107	0.53	0.310	0.875
CCA	**0.0600**	**0.0228**	**0.33**	**0.005**	**0.030**
CTA	0.5301	0.5752	1.03	0.058	0.280

The results were obtained by the logistic regression analysis with adjustment for covariates (age and systolic blood pressure were applied as quantitative variables (value of the trait), while family history of glaucoma, the presence of heart atherosclerosis, heart ischemia, and diabetes (either type I or type II) were used as qualitative variables (yes / no). *OR* odds ratio, *P* significance level, P_{perm} significance level after permutation test Statistically significant values are given in bold.

RP3-337O18.9, SPATA25, SLC12A5, ZNF335) in several tissues/organs (Supplementary Table 5). The 37 SNPs in LD with the four POAG-associated loci are eQTLs ($p_{FDR} \leq 0.05$) affecting transcription of 14 genes (*SPATA25, CD40, ZSWIM1, WFDC3, MMP9, NEURL2, SNX21, PCIF1, RP3-337O18.9, PLTP, RPL13P2, SLC12A5, WFDC10B, ZNF335*) in many organs/tissues (>25) relevant to the POAG pathophysiology (peripheral blood, fibroblasts, endocrine glands, adipose, pituitary, brain, etc.) (Supplementary Table 5). The transcription of several genes may also be affected by the abovementioned clinically-related loci, rs3787268 *MMP9* (*CD40, NEURL2, RP3-337O18.9, PLTP, SLC12A5*) and rs1799750 *MMP1* (*MMP1, MMP10, WTAPP1*) (Supplementary Table 5). Besides, rs3787268 *MMP9* is in strong LD with 12 eQTL SNPs (*CD40, NEURL2, RP3-337O18.9, PLTP, SLC12A5*) (Supplementary Table 5). The POAG-associated SNPs were the splicing quantitative trait loci for three genes and their 36 proxy SNPs affecting sQTL of six genes (*SNX21, ACOT8, SLC35C2, CD40, SLC12A5, PLTP*) in various tissues/organs ($p_{FDR} \leq 0.05$) (this information is given in Supplementary Table 6). The loci associated with the POAG-related clinical traits (rs3787268 *MMP9* and rs1799750 *MMP1*) and SNPs linked to them also possess sQTL value (Supplementary Table 6).

Discussion

This study reports for the very first time the association of polymorphic loci of the *MMP9* and *MMP1* genes with POAG and POAG-related clinical phenotypes in Caucasians from Central Russia.

One of the POAG-associated genetic variants, rs2250889, was previously reported as a candidate SNP for PACG.[21,51] Importantly, the risk allele of this locus for PACG is G, i.e., the same as was identified in our study for POAG. The alternative allele A of this locus was associated with the lower intraocular pressure in POAG patients within haplotype AAC [rs17576-rs3787268- rs2250889] in the present study. Contrary to that, Zhao et al.[21] did not determine any association of rs2250889 *MMP9* with POAG in Chinese.

Association of the rs3918242 *MMP9* loci with POAG has been examined by several studies during the last decade.[14,15,18–21] Three of these studies reported such association in different ethnic populations, Poles,[14] Indians,[19] and Chinese.[21] All these studies identified allele T of the polymorphism as a risk factor. This is in general agreement with our results, which report the alternative allele of this polymorphism, C, as a protective factor for POAG in Caucasians from Central Russia. In contrast, no association of rs3918242 with POAG was found in the Pakistani cohort[15] and by two meta-analyses.[18,20]

The only study of rs3918249 *MMP9* and POAG conducted so far failed to find any association between them in the Chinese population.[21] Therefore, the results of the present study about such association in the Caucasians are novel. However, one should keep in mind that these differences in the results may be explained by the different population-genetic structures of the ethnicities.[52]

Two studies conducted on Pakistani[15] and Chinese[21] reported allele G*rs17576 polymorphism is a risk factor for POAG. The results of the present study are in agreement with the above: alternative allele A*rs17576 was determined as protective for POAG within the CCA haplotype [rs3918242-rs3918249-rs17576]. The protective effect of the allele may be related to lower intraocular pressure determined in POAG patients with haplotypes AA [rs17576-rs3787268] and AAC [rs17576-rs3787268-rs2250889]. However, the data about the probable association of the rs17576 polymorphism with POAG remain inconsistent: the other studies did not find any.[12,18]

This study determined that allele A*rs3787268 *MMP9* was associated with lower intraocular pressure in POAG patients. However, the allele was not associated with the risk for the disease. The recent study on the Chinese sample reported similar results.[21] On the other hand, two other studies on Chinese reported a major allele of this locus, G, as a risk factor for another form of glaucoma, PACG.[21,51] This is in broad agreement with our results about the protective effect of the minor allele A for POAG in Caucasians from Central Russia.

The mechanism by which common variants of the *MMP9* gene contribute to glaucoma predisposition is not known.[21] It was suggested that this mechanism might be related to the elevated expression of metalloproteinases.[53] Indeed, the increased MMP9 expression level was shown to promote the loss of retinal ganglion cells (RGC).[54] Chintala et al.[54] documented that MMP9 is constitutively underexpressed in RGC. Guo et al.[55] reported that the increase in the MMP9 activity in RGC was associated with a reduced amount of laminin, thus indicating the

Table 5. Associations of the studied SNPs with some clinically significant parameters in POAG patients.

				Additive model			Dominant model			Recessive model		
SNP	Gene	Minor allele	n	β	SE	P	β	SE	P	β	SE	P
Intraocular pressure												
rs1799750	*MMP1*	2G	197	0.083	0.068	0.223	0.134	0.108	0.218	0.093	0.1203	0.437
rs679620	*MMP3*	T	201	−0.152	0.068	0.066	−0.167	0.109	0.127	−0.251	0.1166	0.062
rs3918242	*MMP9*	T	200	−0.017	0.096	0.857	0.018	0.109	0.865	−0.351	0.3198	0.273
rs3918249	*MMP9*	C	206	−0.077	0.071	0.279	−0.151	0.100	0.132	−0.006	0.1406	0.962
rs17576	*MMP9*	G	198	−0.058	0.073	0.426	−0.153	0.109	0.161	0.035	0.1352	0.792
rs3787268	*MMP9*	A	207	**−0.176**	**0.083**	**0.036**	**−0.272**	**0.103**	**0.008**	0.010	0.2207	0.962
rs2250889	*MMP9*	G	205	−0.103	0.096	0.282	−0.172	0.114	0.132	0.150	0.2825	0.594
rs17577	*MMP9*	A	205	0.072	0.082	0.380	0.120	0.101	0.235	−0.050	0.2165	0.814
Age of POAG manifestation												
rs1799750	*MMP1*	2G	197	**−0.112**	**0.040**	**0.007**	**−0.218**	**0.064**	**0.0008**	−0.079	0.073	0.282
rs679620	*MMP3*	T	201	0.004	0.042	0.925	−0.042	0.068	0.535	0.060	0.072	0.410
rs3918242	*MMP9*	T	200	0.005	0.060	0.928	−0.019	0.068	0.774	0.227	0.199	0.255
rs3918249	*MMP9*	C	206	0.001	0.044	0.996	−0.039	0.062	0.526	0.078	0.087	0.372
rs17576	*MMP9*	G	198	0.034	0.045	0.445	0.001	0.067	0.993	0.115	0.082	0.164
rs3787268	*MMP9*	A	207	−0.004	0.052	0.941	0.002	0.065	0.975	−0.035	0.138	0.798
rs2250889	*MMP9*	G	205	−0.036	0.061	0.548	−0.029	0.072	0.688	−0.139	0.178	0.437
rs17577	*MMP9*	A	205	−0.074	0.051	0.148	−0.122	0.062	0.053	0.045	0.135	0.739
Systolic blood pressure												
rs1799750	*MMP1*	2G	197	−0.061	0.062	0.325	−0.121	0.099	0.223	−0.041	0.110	0.706
rs679620	*MMP3*	T	201	−0.038	0.063	0.542	−0.101	0.101	0.314	0.004	0.108	0.969
rs3918242	*MMP9*	T	200	0.005	0.089	0.950	0.041	0.101	0.682	−0.292	0.295	0.323
rs3918249	*MMP9*	C	206	−0.068	0.065	0.292	−0.136	0.092	0.141	−0.004	0.129	0.972
rs17576	*MMP9*	G	198	−0.047	0.067	0.482	−0.150	0.100	0.135	0.069	0.124	0.578
rs3787268	*MMP9*	A	207	−0.023	0.078	0.766	−0.124	0.096	0.196	0.393	0.201	0.056
rs2250889	*MMP9*	G	205	0.062	0.090	0.489	0.060	0.107	0.575	0.173	0.265	0.513
rs17577	*MMP9*	A	205	−0.046	0.076	0.541	−0.062	0.093	0.506	−0.037	0.200	0.852

All results were obtained after adjustment for covariates (age and systolic blood pressure (except the analysis of the namesake parameter) were applied as quantitative variables (value of the trait), while family history of glaucoma, the presence of heart atherosclerosis, heart ischemia, and diabetes (either type I or type II) were used as qualitative variables (yes / no)). *SNP*, single nucleotide polymorphism; n, the number of studied POAG patients; *β*, coefficient of the linear regression; *SE*, standard error; *P*, significance level. Statistically significant values are given in bold.

increased ECM degradation. Markiewicz et al.[53] documented the increased mRNA expression of the *MMP-1*, *−9*, *−12*, *interleukin 1β* (*IL-1β*) in POAG patients as compared to the controls. The increased expression of the MMP1, MMP9, MMP12, and IL-1*β* proteins was determined in the aqueous humor of POAG patients. Importantly, allele C*rs3918242 *MMP9* locus (C > T) manifested only 21.86% of the T allele transcriptional activity.[53] Chen et al.[20] observed elevated levels of MMP9 in the blood plasma of POAG patients as compared to healthy controls, as well as the higher plasma levels of the MMP9 mutant alleles of rs3918242, rs2250889, and rs17576 loci as compared with their respective wild type. Overall, these studies suggest that high MMP9 expression (including that related to *MMP9* gene polymorphisms) may contribute to the development of POAG.

MMP9 is one of the tightly regulated families of zinc-dependent enzymes and is implicated in remodeling and homeostasis of ECM.[56] The extracellular matrix pathway has been previously implicated in optic nerve degeneration in GWAS of POAG.[9,10] Recent comprehensive bioinformatics analysis of the genes having been reported as candidates for POAG (termed "POAGome") yielded a high probability for the "Extracellular Matrix Organization" and "Activation of Matrix Metalloproteinases" pathways to be involved in the POAG development.[57]

Previous studies have demonstrated the role of the 2G*rs1799750 *MMP1* allele as a risk factor for both POAG,[13–15,17,20] PACG,[15,17] and exfoliation glaucoma.[17,27] According to our data, this allele is also associated with an earlier age of disease manifestation. However, we did not find a relationship between this locus and the risk of POAG in Caucasians from Central Russia. Several other studies conducted on European populations (Greece, Austria) found no associations of this SNP with POAG[12] or exfoliation glaucoma.[12,58]

The existing inconsistency in the results of the gene association studies of POAG (and other types of glaucoma) prompts further efforts to determine the genetic basis of the disease in different ethnic populations.

The results of the *in silico* analysis of the POAG-associated SNPs suggest that the candidate genes and their proxies with pronounced eQTL and sQTL values may be involved in biological pathways contributing to the POAG pathogenesis. For example, the *ACOT8* and *PLTP* genes play an important role in lipid metabolism. The product of the *ACOT8* gene is acyl-CoA thioesterase 8 involved in the oxidation of fatty acids: hydrolysis of acyl-CoAs to the free fatty acid and coenzyme A.[59] This enzyme was found in almost all cellular compartments such as the endoplasmic reticulum, cytosol, mitochondria, peroxisomes.[59] The *PLTP* gene encodes the lipid transfer protein, which transfers lipopolysaccharides, α-tocopherol, diacylglycerol, cerebroside and may contribute to the development of metabolic syndrome, insulin resistance, obesity, atherosclerosis, and type II diabetes, etc..[60] There is evidence that systemic comorbidities associated with hyperglycemia, hyperlipidemia, endothelial lesions, and atherosclerosis can lead to damage to the retinal nerve fiber layer and the underlying conjunctive tissue.[1,61,62] Besides, PLTP may be involved in signal transduction pathways in human neurons[63] and may contribute to maintaining integrity of the blood-brain barrier, possibly through its involvement in transfer of vitamin E and modulation of the cerebrovascular oxidative stress.[64] One more candidate gene for POAG suggested by the *in silico* analysis, *SLC12A5*, modulates Ca^{2+}-dependent insulin secretion.[65] *SLC12A5* is thought to be *"neuron-specific"* and encodes K^{+}-Cl^{-} cotransporter 2 (KCC2), which extrudes Cl^{-} from neurons and renders the inhibitory function of the neurotransmitters γ-aminobutyric acid (GABA) and glycine.[66] KCC2 dysfunction was implicated in multiple central and peripheral nervous system disorders by disrupting inhibition and causing the collapse of the excitation-inhibition balance.[66] In addition to the above role in the nervous system, *SLC12A5* is expressed in the endocrine cells of the pancreatic islet (glucagon secreting α-cells, insulin-secreting β-cells) and plays an important role in the insulin secretory response.[65]

The *in silico* analysis has been extensively used to determine possible biological pathways underlying the observed genetic associations.[67,68] It allows one to overcome intrinsic limitations of the gene association study design (e.g. absence of "wet" experiments to validate the determined associations), to obtain more complete picture of gene-gene and gene-environment interactions contributing to the trait of interest, and to get insight into possible functional assignments of candidate genes. Besides, it helps to identify possible target genes/SNPs for further experimental validation.[69]

In the recent study, we reported that SNPs rs3918249 and rs2250889 *MMP9* might be the risk variants for exfoliation glaucoma: allele C*rs3918249 decreased the risk for disease (OR = 0.75) while allele G*rs2250889 increased the risk of the disorder (ORs = 1.59–1.68) in Caucasians of the central region of Russia.[25] Along with the results of the present study, this suggests the similar direction and size effect of rs2250889 for both POAG and exfoliation glaucoma in the studied Caucasian cohort of the Central region of Russia.

The results of the present study along with our previous report[25] suggest that the *MMP9* gene may be one of the syntropic genes for POAG and PXFG. The other genes manifesting the syntropic effect for these diseases are *CDKN2B-AS1*[70,71] and *LOXL1*.[72–75]

One limitation of this study should be acknowledged though. Similar to other association studies, it did not analyze both gene and protein expression. Therefore, the obtained results should be interpreted with caution.

In summary, allele G*rs2250889 *MMP9* elevated the risk for POAG while haplotype CCA [rs3918242-rs3918249-rs17576] of the *MMP9* decreased the risk for POAG in the Caucasian population of the central region of Russia. The four POAG-associated loci of the *MMP9* gene and their 38 proxy SNPs have epigenetic potential affecting transcription of 14 genes and alternative splicing of six genes in many tissues/organs relevant to the POAG pathophysiology. Locus rs3787268 *MMP9*, haplotypes AA [rs17576-rs3787268] and AAC [rs17576-rs3787268-rs2250889] of the *MMP9* gene were associated with low intraocular pressure in POAG patients, and rs1799750 *MMP1* was associated with earlier age of the disease manifestation.

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