• Basic Research •

Genes of tumor necrosis factors and their receptors and the primary open angle glaucoma in the population of Central Russia

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Abstract

• AIM: To examine the association of genetic polymorphisms (-308)G/A *TNFα*, (+250)A/G *Ltα*, (+36)A/G *TNFR1*, (+1663) A/G *TNFR2* with the development of primary open angle glaucoma (POAG) among people in Central Russia.

• METHODS: The study sample included 443 individuals, of which 252 patients with POAG and 191 individuals in the control group. Genotyping of (-308)G/A *TNFα*, (+250)A/G *Ltα*, (+36)A/G *TNFR1*, (+1663)A/G *TNFR2* was performed using polymerase chain reaction. The distribution of alleles and genotypes of the studied DNA markers in the groups was examined by 2×2 contingency tables and χ^2 with the Yates's correction for continuity and odds ratios (OR) with 95% confidence intervals (CI).

• RESULTS: Allele (-308)G *TNFα* (*P*=0.01, OR=1.78, 95%CI 1.12-2.85) was identified as a risk factor for POAG. Homozygotes (-308) AA *TNFα* are at a lowest risk for development of the disease (*P*=0.01, OR=0.0005). The following combination of genetic variants of cytokines were associated with a reduced risk of POAG: (+1663)A *TNFR2* and (+250)G *Ltα* (OR=0.34)

• CONCLUSION: Genetic polymorphisms (-308)G/A *TNFα*, (+250)A/G *Ltα*, (+1663)A/G *TNFR2* associated with the development of POAG in the population of Central Russia.

• KEYWORDS: primary open angle glaucoma; tumor necrosis factor; tumor necrosis factor receptor; gene polymorphism

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INTRODUCTION

Glaucoma is a heterogeneous group of diseases characterized by progressive optic neuropathy and the typical reduction in visual function, being the second leading cause of irreversible blindness[1]. Primary open angle glaucoma (POAG) is the most prevalent clinical form of the disease accounting for 90% of all forms of glaucoma[2].

Currently, the etiopathogenic significance in POAG is thought to be due to violations in apoptosis. Tumor necrosis factors and their receptors are among key elements in this process[3-4]. Therefore, several genetic polymorphisms of tumor necrosis factors and their receptors have been analyzed for their possible association with POAG[5-11]. Among those, (-308) G/A *TNFα* (guanine to adenine substitution at position 308 of the *TNFα* promoter region) was the most commonly studied. The data on its association with the development of POAG were contradictory. For example, while in some populations no association of this polymorphism with the emergence of POAG was found[12-15], such an association was reported in populations from China, Iran, Egypt and Saudi Arabia[5,7,16-17].

In this study, we analyzed four polymorphisms of cytokines, (-308)G/A *TNFα*, (+250)A/G *Ltα*, (+36)A/G *TNFR1*, and (+1663)A/G *TNFR2*, for their possible association with development of POAG in the population of Central Russia.

SUBJECTS AND METHODS

The total study sample consisted of 443 participants, including 252 patients with POAG and 191 individuals in the control group. The patients with POAG were enrolled in the study according to the following criteria: open anterior chamber angle, increased intraocular pressure (≥21 mm Hg), characteristic changes in the optic disc (notching, neuroretinal rim thinning, increased the ratio of excavation/optic disc), visual field defects characteristic of glaucoma (arcuate scotoma, narrowing of the field of view with the nose, paracentral scotoma) and the absence of conditions leading to secondary glaucoma.

Table 1 SNP function prediction of the studied polymorphisms

SNP	Allele	TFBS	Splicing (ESE or ESS)	miRNA (miRanda)	RP	Conservation	Allele frequency	
							Allele	European
rs1800629	A/G	+	-	-	0.04	0	G	0.795
rs909253	G/A	+	-	-	0.21	0	A	0.649
rs767455	C/T	-	+	-	0.52	1	T	0.505
rs1061624	A/G	-	-	+	0.24	0.001	A	0.541

Data is obtained using the online program from the site the National Institute of Environmental Health Sciences (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html). TFBS: Transcription factor binding site; ESE: Exonic splicing enhancer; ESS: Exonic splicing silencer; RP: Regulatory potential.

Table 2 Primers and probes used to genotype the studied polymorphisms

Polymorphisms	Position in the gene	5'-3' sequence of the primers and probes	References
-308G/A *TNFα* (rs1800629)	Promotor	F: 5'-GAAATGGAGGCAATAGGTTTTGAG-3' R: 5'-GGCCACTGACTGATTTGTGTGTAG-3' 5'-FAM-CCGTCCTCATGCC- RTQ1-3' 5'-ROX-CCGTCCCCATGCC - RTQ1-3'	Landi *et al*[19]
+250A/G *Ltα* (rs909253)	Intron 1	F: 5'-CAGTCTCATTGTCTCTGTCACACATT-3' R: 5'-ACAGAGAGAGACAGGAAGGGAACA-3' 5'-FAM:CCATGGTTCCTCTC-RTQ1-3' 5'-ROX:CTGCCATGATTCC-RTQ1-3'	de Jong *et al*[20]
+36A/G *TNFR1* (rs767455)	Exon 1	F: 5'- AGCCCACTCTTCCCTTTGTC-3' R: 5'-CCACCGTGCCTGACCTG-3' 5'- FAM: CTGCTGCCACTGGT-RTQ1 -3' 5'- ROX: CTGCTGCCGCTGGT-BHQ2 -3'	Chae *et al*[21]
+1663A/G *TNFR2* (rs1061624)	3'UTR	F: 5'- TGACCTGCAGGCCAAGAG-3' F: 5'- CCATGGCAGCAGAGGCTTT-3' 5'-FAM: CACAACCCGCTGCC - RTQ1-3' 5'-ROX: CCACAACTCGCTGCC - BHQ2-3'	Ferguson *et al*[22]

The control group included individuals without POAG, acute diseases of the eye at the time of the survey, or any somatic pathology resulting in secondary injury to the eyes. All study participants signed a written informed consent in accordance with the principles of the Helsinki Declaration. The participants were examined at the Department of Eye Microsurgery of St. Iasaf Belgorod Regional Clinical Hospital.

All participants were genotyped for the following polymorphisms: (-308)G/A of *TNFα* (rs1800629), (+250)A/G of *Ltα* (rs909253), (+36)A/G of *TNFR1* (rs767455) and (+1663)A/G of *TNFR2* (rs1061624). There are data about association of *TNFα* (rs1800629) and *Ltα* (rs909253) with POAG in populations from China, Iran, Egypt and Saudi Arabia)[5,7,16-17]. *TNFR1* and *TNFR2* encode the respective receptors. Furthermore, the selected polymorphisms may be of functional significance. Specifically, according to the National Institute of Environmental Health Sciences (NIEHS) (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html), rs767455 is located at the splicing site and has high regulatory potential (0.52), rs1061624 is located in the miRNA-binding site, both rs909253 and rs1800629 are located at the transcription factor binding sites (Table 1). Yet, rs1800629, rs909253, and rs1061624 are tag single nucleotide polymorphism (SNPs).

Genomic DNA was isolated from blood drawn from cubital vein of a proband. The protocol of DNA isolation was described elsewhere[18]. The studied loci were genotyped using TaqMan probes and primers described previously (Table 2).

Table 3 Physical characteristics of the subjects from the case and control groups

Characteristics	Cases	Controls
Total	252	191
Age, a (min-max)	70.53±8.43 (46.0-89.0)[a]	69.24±10.14 (40.0-87.0)
BMI	28.12±4.84[a]	29.04±6.01
Males	125 (49.6%)[a]	93 (48.7%)
Females	127 (50.4%)[a]	98 (51.3%)

BMI: Body mass index; [a]P>0.05.

The allele frequencies were analyzed for their correspondence to the Hardy-Weinberg equilibrium (HWE) using the χ^2 criterion. The distribution of alleles and genotypes in groups was assessed using 2×2 contingency tables, odds ratios (OR) with 95% confidence intervals (CI), and the Yates's χ^2 test. A contribution of the genetic variants and their combinations POAG was analyzed, using the MCMC and Bayesian nonparametric statistics methods suggested by Favorov *et al*[23] and implemented in APSampler. The error of the first kind from multiple comparisons was corrected by a permutation test (p_{perm})[24].

RESULTS

The physical characteristics of the participants are given in Table 3. The control group is similar to the case group by

Table 4 Comorbidities in the studied groups

Comorbidity (if any)	POAG patients		Controls		χ^2 (*P*)
	n	%	*n*	%	
Absence of comorbidity	19	7.54	25	13.09	3.15 (0.08)
Hypertonia	162	64.29	120	62.83	0.05 (0.83)
Arterial hypotension	8	3.17	10	5.24	0.71 (0.40)
Heart atherosclerosis	55	21.83	41	21.47	0.01 (1.00)
Heart ischemia	112	44.44	78	40.84	0.44 (0.51)
Other heart pathology	11	4.37	13	6.81	0.83 (0.36)
Diabetes					
Type I	2	0.79	1	0.52	0.01 (1.00)
Type II	38	15.08	27	14.14	0.02 (0.89)
Other endocrine disorders	13	5.16	7	3.66	0.27 (0.60)
Nervous system pathology	21	8.33	24	12.57	1.69 (0.19)
Kidney pathology	6	2.38	3	1.57	0.07 (0.80)
Digestive system pathology	25	9.92	13	6.81	0.98 (0.32)
Respiratory system pathology	9	3.57	3	1.57	0.98 (0.32)
Blood pathology	3	1.19	1	0.52	0.05 (0.82)
Any other pathology	32	12.70	32	16.75	1.14 (0.29)

Table 5 The frequency of genotypes and alleles of the studied polymorphisms in patients with POAG and in the control group

Polymorphisms	Genotypes, alleles	Controls (*n*=191)		POAG patients (*n*=252)		OR (95% CI)	χ^2 (*P*)
		n	%	*n*	%		
-308G/A *TNFα* (rs1800629)	*-308GG*	139	76.80	208	84.21	1.61 (0.96-2.70)	3.28 (0.07)
	-308GA	36	19.89	39	15.79	0.76 (0.45-1.28)	0.95 (0.33)
	-308AA	6	3.31	0	0	0.0005 (-)	6.08 (0.01)
	-308G	314	86.74	455	92.11	1.78 (1.12-2.85)	6.01 (0.01)
	-308A	48	13.26	39	7.89	0.56 (0.35-0.90)	
	$\chi^2_{(HWE)}$ (*P*)	3.32 (>0.05)		1.81 (>0.05)		-	
+36A/G *TNFR1* (rs767455)	*+36AA*	44	24.58	59	23.89	0.96 (0.60-1.55)	0.01 (0.96)
	+36AG	83	46.37	126	51.01	1.20 (0.80-1.80)	0.72 (0.40)
	+36GG	52	29.05	62	25.10	0.82 (0.52-1.29)	0.64 (0.43)
	+36A	171	47.77	244	49.39	1.07 (0.81-1.42)	0.16 (0.69)
	+36G	187	52.23	250	50.61	0.94 (0.71-1.24)	
	$\chi^2_{(HWE)}$ (*P*)	0.90 (>0.05)		0.10 (>0.05)		-	
+1663A/G *TNFR2* (rs1061624)	*+1663AA*	36	20.57	38	15.32	0.70 (0.41-1.19)	1.61 (0.21)
	+1663AG	75	42.86	128	51.61	1.42 (0.95-2.14)	2.81 (0.09)
	+1663GG	64	36.57	82	33.07	0.86 (0.56-1.31)	0.41 (0.52)
	+1663A	147	42.00	204	41.13	0.97 (0.72-1.29)	0.03 (0.86)
	+1663G	203	58.00	292	58.87	1.04 (0.78-1.38)	
	$\chi^2_{(HWE)}$ (*P*)	2.53 (>0.05)		1.07 (>0.05)		-	
+250A/G *Ltα* (rs909253)	*+250AA*	85	46.96	139	55.83	1.43 (0.95-2.14)	2.95 (0.09)
	+250AG	81	44.75	89	35.74	0.69 (0.46-1.04)	3.19 (0.07)
	+250GG	15	8.29	21	8.43	1.02 (0.49-2.15)	0.01 (1.00)
	+250A	251	69.34	367	73.69	1.24 (0.91-1.69)	1.76 (0.19)
	+250G	111	30.66	131	26.31	0.81 (0.59-1.10)	
	$\chi^2_{(HWE)}$ (*P*)	0.50 (>0.05)		1.52 (>0.05)		-	

HWE: Hardy-Weinberg equilibrium.

gender, age and prevalence of various comorbidities (Table 4). Allele (-308)G *TNFα* was associated with an increased risk for POAG (*P*=0.01, OR=1.78, 95% CI 1.12-2.85) (Power 0.72), whereas homozygotes (-308)AA *TNFα* had a lower risk for development of POAG (*P*=0.01, OR=0.0005, 95% CI -) (Power 0.79) (Table 5).

The combination of the following genetic variants also showed association with a lower risk of POAG: (+1663)A *TNFR*2 and (+250)G *Ltα* (Case 5.65%, Control 15.12%, *P*=0.001, p_{perm}=0.0003, OR=0.34, 95% CI 0.17-0.66).

DISCUSSION

The study identified the risk and protective genetic variants for POAG. Along with the statistically significant association of individual SNPs, *i.e.* (-308)G *TNFα* is a risk factor for POAG and (-308)AA *TNFα* as a protective factor, a significant contribution to the combination of genetic variants (+1663) A *TNFR*2 and (+250)G *Ltα* in lowering a risk for POAG was found.

The role of tumor necrosis factors in POAG is of a great interest, because they contribute to development of optic neuropathy. *TNFα*, binding to *TNFR1*, either directly induces apoptosis of retinal ganglion cells or indirectly participates in the progression of glaucomatous neurodegeneration. Elevated intraocular pressure and vascular factors leads to the activation of astrocytes that produce *TNFα*, which in turn initiates the process of cell death. Moreover, activated astrocytes produce nitric oxide synthase (NOS-2) and thereby affect the formation of NO. Hyperproduction of NO and its transformation into peroxynitrite also induce apoptosis of retinal ganglion cells[3-4]. Thus, tumor necrosis factors may be involved in the pathogenesis of POAG as the main mediators of apoptosis, but the exact mechanism of the involvement of these cytokines in developing POAG remains unclear.

The available data about a probable role of the studied polymorphisms in development of POAG are inconsistent. For example, Chinese researchers determined higher frequency of allele G of the (-308)G/A *TNFα* polymorphism in POAG patients as compared to the controls (OR=1.89, 95% CI 1.14-3.13)[6], which is in agreement with our results. On the other hand, several studies reported that another allele, (-308)A *TNFα*, is a risk factor for POAG[5,7,11]. Finally, some studies did not found any association between the (-308)G/A *TNFα* polymorphism and POAG[9-10].

The data about possible contribution of (+250)A/G *Ltα*, (+36) A/G *TNFR1* and (+1663)A/G *TNFR*2 to POAG are extremely scarce. Al-Dabbagh *et al*[7] reported that frequency of genotype +250GG *Ltα* was higher in the sample of 200 POAG patients from Saudi Arabia (*P*=0.001, OR=3.28), whereas genotype +250AG *Ltα* was more common in the controls (*P*=0.001, OR=0.33). These results differ from ours, which may be due to the different population genetic structure and/or study design. One study determined no association of the +36A/G *TNFR1* locus with development of POAG[5]. No studies on association of the (+1663)A/G *TNFR2* polymorphism with POAG was found in the available literature.

In summary, the results of the present study suggest that allele (-308)G *TNFα* is a risk factor for POAG in a population of Central Russia, whereas carriers of the (-308)AA *TNFα* genotype and haplotype (+1663)A *TNFR2*/(+250)G *Ltα* (OR=0.34) have a lower risk to develop POAG.

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