• Basic Research •

TNF-α gene polymorphisms: association with age-related macular degeneration in Russian population

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Abstract

• AIM: To study polymorphisms in promotor regions of *tumor necrosis factor (TNF)-α TNF-863A/C* (rs1800630), *TNF-308A/G* (rs1800629), and *TNF-238A/G* (rs361525) in patients with age-related macular degeneration (AMD) and associations of complex *TNF-α* genotypes with AMD.

• METHODS: One hundred and two patients (82 women, 20 men; mean age 64.2±1.2y) with AMD and 100 healthy age- and sex-matched controls (82 women, 18 men; 60±1.4y) were included in the study. All subjects were Caucasian, all subjects and their parents were inhabitants of Russia. Genomic DNA was obtained from EDTA-preserved blood using the standard phenol-chloroform method. Polymorphisms were detected by polymerase chain reaction followed by the restriction fragment length polymorphism method. The following *TNF-α* genotypes were studied: *TNF-α-238 AA, GA, GG, TNF-α-308 AA, GA, GG, TNF-α-863 AA, CA, CC.*

• RESULTS: Differences in *TNF-α-863 and TNF-α-238* genotypes frequencies in patients with AMD and healthy controls were not found. The distribution of *TNF-α-308 AA* and *TNF-α-308 GA* genotypes was significantly different between the studied group and the controls [odds ratios (OR) =0.22, *P*=0.0287 and OR=2.91, *P*=0.0063, respectively]. *TNF-863CC/TNF-308GA* and *TNF-308GA/TNF-238GG* genotypes were associated with the increased risk of AMD (OR=2.48, *P*=0.0332 and OR=2.51, *P*=0.0187, respectively). Five genotypes combinations appeared to be protective.

• CONCLUSION: In the present study, single nucleotide polymorphisms and complex polymorphisms of one of the key inflammatory cytokines TNF-α, and a number of significant associations of these polymorphisms with AMD in Russian population have been shown. Complex analysis of genotypes could be important in AMD risk factors detection and studying pathogenesis.

• **KEYWORDS:** *tumor necrosis factor-a*; genetic polymorphisms; age-related macular degeneration
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INTRODUCTION

Age-related macular degeneration (AMD), the leading cause of irreversible vision loss worldwide, is a complex disease caused by multiple environmental and genetic risk factors. AMD is caused by a combination of genetic, environmental and lifestyle risk factors. Researchers have identified more than 20 genes loci influencing the risk of AMD[1-3].

Recent studies have shown the role of immune system in AMD development and progression[4]. Increased concentrations of a number of inflammatory cytokines have been found both in serum and locally in ocular tissues or fluids in patients with AMD[5]. AMD is shown to be associated with several single nucleotide polymorphisms (SNPs) in genes, many of which are encoding cytokines. Pro-inflammatory cytokine tumor necrosis factor (TNF)-α plays an important role in immune response regulation. The role of *TNF-α* gene polymorphisms in AMD has been investigated[6-7].

The transcription of *TNF-α* is genetically regulated, and recent studies have shown that promoter polymorphisms at -238 (rs361525), -308 (rs1800629) and -863 (rs1800630) positions of its gene could regulate *TNF-α* production[8-10]. These genetic polymorphisms may have implications to AMD pathogenesis due to inflammatory processes imbalance caused by TNF-α production dysregulation.

The purpose of the present paper was to study *TNF-α* gene polymorphisms in patients with AMD in comparison to patients without AMD in Russian (Caucasian) population and to study associations of complex *TNF-α* genotypes with AMD.

Table 1 Characteristics of RFLP-PCR primers, restriction endonucleases and product size

Polymorphic positions	Primer sequence	Restriction enzyme	Product size (bp)	
			Wild-type	Minore-type
TNF-a-863 C→A	5' GGCTCTGAGGAATGGGTTAC 3' 5' CTACATGGCCCTGTCTTCGTTACG 3'	BstBAI	125	102; 23
TNF-a-308 G→A	5' AGGCAATAGGTTTTGAGGGCCAT 3' 5' ACACTCCCCATCCTCCCGGCT 3'	Bsp19I	97; 20	118
TNF-a-238 G→A	5' AGAAGACCCCCCTCGGAACC 3' 5' ATCTGGAGGAAGCGGTAGTG 3'	Msp I	132; 20	152

SUBJECTS AND METHODS

Ethical Approval The study was conducted at the Novosibirsk Branch of the Academician S.N. Fyodorov Eye Microsurgery Federal State Institution, Novosibirsk, Russia. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the aforementioned institution. All patients signed an informed consent form prior to participation in the study.

Patients One hundred and two patients (82 women, 20 men; mean age 64.2±1.2y) with AMD and one hundred healthy age- and sex-matched controls (82 women, 18 men; 60±1.4y) were included to the study. All subjects were Caucasian; all of the subjects and their parents were inhabitants of Russia. Diagnosis of AMD was based on the standard ophthalmological methods: visual acuity measurement, intraocular pressure measurement (tonometry), biomicroscopy, eye fundus examination (ophthalmoscopy), fields of vision measurement (perimetry), and optical coherence tomography.

Patients included to the study had been diagnosed with AMD at least 3y prior to the inclusion (6±3.9y; range from 3 to 10y). At the time of inclusion there were 32 patients (31.4%) with age-related eye disease study (AREDS) stage 2 and 70 patients (68.6%) with AREDS stage 3 AMD. The frequency of smoking being a strong factor for AMD was comparable: 35 patients (34.3%) in the study group and 39 patients (39%) in the control group.

Exclusion criteria for participation in the study were: acute or exacerbation of chronic inflammatory ocular diseases, glaucoma, uveitis, complicated cataract, retinal detachment, rubeosis iridis; diabetes mellitus, autoimmune diseases, tumors of any localization. Patients with chronic heart failure, chronic renal failure and acute or exacerbations of chronic systemic diseases were also not included to the study.

The primary inclusion of the patients to the study group was based on the diagnosis of AMD; they were randomly selected from the internal registry of the clinic. Control group was formed from patients underwent prophylactic examination in the clinic. At this stage, 178 patients were enrolled to the study group, 159 patients to the control group. Subsequently, during clinical examination (including anamnesis collection, medical history analysis with special attention to non-ophthalmological diseases) and ophthalmological examination, based on the exclusion criteria, 76 patients (43%) were excluded from the study group and 59 patients (37%) from the control group.

Polymorphisms Detection Genomic deoxyribonucleic acid (DNA) was obtained from ethylene-diamine-tetraacetic-acid (EDTA)-preserved blood from patients and control subjects using the standard phenol-chloroform method. Blood samples were kept at -20℃ before laboratory testing, and assessed within 2mo after the collection.

Polymorphisms were detected by polymerase chain reaction (PCR) followed by the restriction fragment length polymorphism (RFLP) method. PCR was carried out using a MyCycler™ Thermal Cycler System (Bio Rad, USA). The 20 μL reaction solution contained one unit of TaqDNA polymerase (SibEnzyme, Novosibirsk, Russia), 0.5 μmol/L of each primer, 0.25 mmol/L of each desoxynucleoside-triphosphate, and 50-200 ng of genomic DNA. Reaction buffer added to DNA polymerase contained 60 mmol/L of Tris-HCl (pH 8.5, 25℃), 1.5 mmol/L $MgCl_2$, 25 mmol/L KCl, 10 mmol/L 2-mercaptoethanol, and 0.1% Triton X-100. Applied TNF PCR program was at 95℃ for 5min, followed by 40 cycles of denaturation at 95℃ for 30s, primer annealing at 59℃ for 30s, and process extension at 72℃ for 40s. Final extension step was carried out at 72℃ for 3min. Amplification products were exposed to respective restriction enzymes (1-2 activity units) in 20 μL volume. After enzymatic digestion PCR products were size separated and visualized in 2.5% agarose gel. Specific primers used for amplification of *TNF-α* promoter region at position -238G/A, -308G/A, and -863C/A, restriction enzymes (SibEnzyme Ltd., Novosibirsk, Russia) and products size are shown in Table 1. Primers specific for *TNF-α* gene sequences were synthesized by SibEnzyme (Novosibirsk, Russia).

Statistical Analysis We used Chi-squared test to find out if genotype frequencies distribution in the controls fits to Hardy-Weinberg equilibrium (HWE). HWE is widely used in population genetics as a base for analysis; it states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences[11]. HWE in the controls was tested by comparing the expected and observed genotype frequencies using Pearson's Chi-squared. The analysis was performed to

Table 2 *TNF-α* polymorphisms in patients with AMD compared to the controls *n* (%)

TNF-α genotypes	Genotypes distribution		OR	95%CI	*P*
	Study group	Controls			
-238					
AA	1 (0.98)	1 (1.00)	0.98	0.03-36.40	1.000
GA	11 (10.78)	7 (7.00)	1.61	0.55-4.83	0.486
GG	90 (88.24)	92 (92.00)	0.65	0.23-1.62	0.508
-308					
AA	3 (2.94)	12 (12.00)	0.22	0.05-0.88	0.029
GA	29 (28.43)	12 (12.00)	2.91	1.31-6.54	0.006
GG	70 (68.63)	76 (76.00)	0.71	0.36-1.39	0.365
-863					
AA	0	2 (2.00)	0.00	0.00-4.00	0.244
CA	24 (23.53)	33 (33.00)	0.62	0.32-1.21	0.181
CC	78 (76.47)	65 (65.00)	1.75	0.91-3.39	0.102

TNF-α: Tumor necrosis factor-α; AMD: Age-related macular degeneration.

Table 3 Association of *TNF-α* complex genotypes with AMD *n* (%)

TNF-α genotypes	Genotypes distribution		OR	95%CI	*P*
	Study group	Controls			
TNF-863CC/TNF-308GA	22 (21.57)	10 (10.00)	2.48	1.11-5.54	0.033
TNF-863CC/TNF-308AA	3 (2.94)	11 (11.00)	0.25	0.07-0.91	0.028
TNF-863CA/TNF-308GG	17 (16.67)	30 (30.00)	0.47	0.24-0.92	0.031
TNF-308GA/TNF-238GG	26 (25.49)	12 (12.00)	2.51	1.19-5.31	0.019
TNF-308AA/TNF-238GG	3 (2.94)	12 (12.00)	0.22	0.06-0.81	0.016
TNF-863CC/TNF-308AA/TNF-238GG	3 (2.94)	11 (11.00)	0.25	0.07-0.91	0.028
TNF-863CA/TNF-308GG/TNF-238GG	14 (13.73)	26 (26.00)	0.45	0.22-0.93	0.034

TNF-α: Tumor necrosis factor-α; AMD: Age-related macular degeneration.

determine the possibility of using the group as control. For the groups with less than five patients the differences in *TNF-α* genotypes polymorphism frequencies were analyzed by Chi-squared test with Yates's correction or two-tailed Fisher test. *P* value of <0.05 was accepted as indicating statistical significance. Odds ratios (OR) were calculated with a 95% confidence interval (CI). All calculations were performed using SPSS software, version 13.

RESULTS

In the promoter region of *TNF-α* gene three SNPs were detected: *TNF-α-863* (rs1800630), *TNF-α-308* (rs1800629) and *TNF-α-238* (rs361525). *TNF-α* genotype frequencies are shown in Table 2.

All genotype frequencies in control group were consistent with HWE criteria (*P*>0.05). We did not find any differences in *TNF-α-863* and *TNF-α-238* genotypes frequencies in patients with AMD and healthy controls. The distribution of *TNF-α-308 AA* and *TNF-α-308 GA* genotypes was significantly different between the studied group and the controls (OR=0.22, *P*=0.0287 and OR=2.91, *P*=0.0063, respectively). All SNPs genotypes combinations with more than 1% frequency and associated with high risk of AMD are described in Table 3. *TNF-863CC/TNF-308GA* and *TNF-308GA/TNF-238GG* genotypes were found in 21.57% and 25.49% patients with AMD, respectively, compared to 10.0% and 12.0% in control group, respectively. These SNPs genotype combinations were associated with increased risk of AMD (OR=2.48, *P*=0.0332 and OR=2.51, *P*=0.0187, respectively). On the contrary, five genotypes combinations were found to have lower frequency in AMD patients than in the controls and could be associated with decreased risk of AMD development.

DISCUSSION

Genetic knowledge on AMD has expanded tremendously and the role of inflammation in the development of AMD has become evident[12-13]. Pro-inflammatory cytokine TNF-α, which encoding gene is located on chromosome 6p21.3, is a multifunctional cytokine playing a pivotal role in immune response regulation. The remarkable therapeutic effect of anti-TNF-α agents shown in wet AMD confirms the important role of TNF-α in its pathogenesis[14-15]. To determine possible associations with the disease -863, -308 and -238 polymorphisms of *TNF-α* promoter were studied in AMD patients from Russian population. The choice of polymorphisms positions was based on their location in promoter non-coding region of the gene and influence to TNF-α production[8].

None of the studied genotypes of *TNF-α* gene polymorphisms at *TNF-α-863C/A* and *TNF-α-238 G/A* loci was associated with pathology. Only *-308 G/A TNF-α* gene polymorphism was found to be associated with AMD in the studied population. In contrast, among six candidate SNPs of TNF-α gene (-238 G/A, -308 G/A, +489 G/A, -857 C/T, -863 C/A, and -1031 T/C), only -1031 T/C was significantly associated with wet AMD in the Taiwan Chinese population[6]. Other data indicate that polymorphisms in the -1031T/C and -308G/A *TNF-α* gene do not play an important role in dry AMD in the population from northeastern Iran[7]. This phenomenon is not surprising because studies have suggested that the frequency of genetic markers often shows high variation among various ethnic and racial groups[16-17]. These inconsistencies could also be explained by differences in sample size, methodologies and dominance of distinct etiological factors in different populations.

We suggested that complex analysis of several polymorphic positions of *TNF-a* promoter region could find associations with the disease more clearly. Two *TNF-α* complex genotypes were found to be associated with AMD. The frequency of five genotypes was significantly decreased in the study group compared to patients without AMD which therefore may indicate the protective effect of these genotypes. The molecular mechanism of obtained results is not clear. Using genotypes and haplotypes analysis the association of certain genotypes with various levels of TNF-α production had been earlier demonstrated[18]. The linkage disequilibrium between *TNF-α-238*, *TNF-α-308* and the *TNF-α-863* was not found[19].

The participation of TNF-α in inflammation and its genetic influence on other cytokines plays an important role in the disease progression and outcomes[20-22]. The associations of *TNF-α* genotype with AMD are not absolutely clear that has been shown in different contradictory studies. Nevertheless, it is clear that the genetic regulation of *TNF-α* at polymorphic sites is important for inflammation.

During the inflammation cytokines form a network ("cytokines cascade") with complex interactions of cytokines within this network (*e.g.* a number of cytokines could display synergy, others could act as antagonists). The same "network" is formed by cytokines genes. So we suggest that it could be informative to study complex genetic polymorphisms of different cytokines in patients with AMD to determine their association with the disease. We believe that it could be useful for screening purposes.

In our study a number of significant associations of *TNF-α* gene polymorphisms and AMD have been shown. We detected both complex genotypes associated with the high risk of the disease and carrying protective characteristics. Our data indicate that the TNF-α polymorphism could be associated with AMD in Russian population that could be considered as one of the steps to development of screening instrument.

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REFERENCES

1 Fritsche LG, Igl W, Bailey JN, *et al*. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet* 2016;48(2):134-143.

2 Black JM, Clark SJ. Age-related macular degeneration: genome-wide association studies to translation. *Genet Med* 2016;18(4):283-289.

3 Swaroop A, Chen W, Stambolian D, Branham K, Othman M, Chew E, Heckenlively J, Tosakulwong N, Edwards AO, Abecasis G. Genome wide association study (GWAS) of age-related macular degeneration (AMD). *Invest Ophthalmol Vis Sci* 2009;50(13):1614.

4 Nita M, Grzybowski A, Ascaso FJ, Huerva V. Age-related macular degeneration in the aspect of chronic low-grade inflammation (pathophysiological paraInflammation). *Mediators Inflamm* 2014;2014: 930671.

5 Cao S, Ko A, Partanen M, Pakzad-Vaezi K, Merkur AB, Albiani DA, Kirker AW, Wang A, Cui JZ, Forooghian F, Matsubara JA. Relationship between systemic cytokines and complement factor H Y402H polymorphism in patients with dry age-related macular degeneration. *Am J Ophthalmol* 2013;156(6):1176-1183.

6 Wan L, Lin HJ, Tsai Y, Lee CC, Tsai CH, Tsai FJ, Tsai YY, Lin JM. Tumor necrosis factor-α gene polymorphisms in age-related macular degeneration. *Retina* 2010;30(10):1595-1600.

7 Bonyadi MH, Bonyadi M, Ahmadieh H, Fotuhi N, Shoeibi N, Saadat S, Yagubi Z. Tumor necrosis factor gene polymorphisms in advanced non-exudative age-related macular degeneration. *J Ophthalmic Vis Res* 2015;10(2):155-159.

8 Qidwai T, Khan F. Tumour necrosis factor gene polymorphism and disease prevalence. *Scand J Immunol* 2011;74(6):522-547.

9 El-Tahan RR, Ghoneim AM, El-Mashad N. TNF-α gene polymorphisms and expression. *SpringerPlus* 2016;5(1):1508.

10 Elahi MM, Asotra K, Matata BM, Mastana SS. Tumor necrosis factor alpha-308 gene locus promoter polymorphism: an analysis of association with health and disease. *BBA Mol Basis Dis* 2009;1792(3):163-172.

11 Stark AE. Estimation of divergence from Hardy-Weinberg form. *Twin Res Hum Genet* 2015;18(4):399-405.

12 Parmeggiani F, Sorrentino FS, Romano MR, Costagliola C, Semeraro F, Incorvaia C, D'Angelo S, Perri P, De Nadai K, Roversi EB, Franceschelli P, Sebastiani A, Rubini M. Mechanism of inflammation in age-related macular degeneration: an up-to-date on genetic landmarks. *Mediators Inflamm* 2013;2013:435607.

13 Knickelbein JE, Chan CC, Sen HN, Ferris FL, Nussenblatt RB. Inflammatory mechanisms of age-related macular degeneration. *Int Ophthalmol Clin* 2015;55(3):63-78.

14 Theodossiadis PG, Liarakos VS, Sfikakis PP, Vergados IA, Theodossiadis GP. Intravitreal administration of the anti-tumor necrosis factor agent infliximab for neovascular age-related macular degeneration. *Am J Ophthalmol* 2009;147(5):825-830.

15 Fernández-Vega B, Fernández-Vega Á, Rangel CM, Nicieza J, Villota-Deleu E, Vega JA, Sanchez-Avila RM. Blockade of tumor necrosis factor-alpha: a role for adalimumab in neovascular age-related macular degeneration refractory to anti-angiogenesis therapy? *Case Rep Ophthalmol* 2016;7(1):154-162.

16 Winham SJ, de Andrade M, Miller VM. Genetics of cardiovascular disease: importance of sex and ethnicity. *Atherosclerosis* 2015;241(1): 219-228.

17 Jing L, Su L, Ring BZ. Ethnic background and genetic variation in the evaluation of cancer risk: a systematic review. *PLoS One* 2014;9(6):e97522.

18 Kazzi SN, Tromp G, Quasney MW, Buhimschi IA. Haplotypes of tumor necrosis factor gene and tracheal aspirate fluid levels of tumor necrosis factor-α in preterm infants. *Pediatr Res* 2008;64(2):165-170.

19 Tan JH, Temple SE, Kee C, Waterer GW, Tan CR, Gut I, Price P. Characterisation of TNF block haplotypes affecting the production of TNF and LTA. *Tissue Antigens* 2011;77(2):100-106.

20 Newton K**,** Dixit VM. Signaling in innate immunity and inflammation**.** *Cold Spring Harb Perspect Biol* 2012;4(3):a006049.

21 Woo HJ, Yu C, Kumar K, Gold B, Reifman J. Genotype distribution-based inference of collective effects in genome-wide association studies: insights to age-related macular degeneration disease mechanism. *BMC Genomics* 2016;17:695.

22 Tsiloulis AN, Zacharaki F, Kotoula MG, Chatzoulis D, Morrison MA, Mayne K, Dardiotis E, Stefanidis I, Almpanidou P, DeAngelis MM, Tsironi EE. Genetic variants in complement pathway and *ARMS2/HTRA1* genes and risk of age-related macular degeneration in a homogeneous population from central Greece. *Ophthalmic Genet* 2016;37(3):339-344.