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

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Abstract

Aim: To study polymorphisms in promoter 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, GA, 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).

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-α; 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[3]. 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[6,10]. 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.
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, rubositis 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°C 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 20μL 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°C), 1.5 mmol/L MgCl₂, 25 mmol/L KCl, 10 mmol/L 2-mercaptoethanol, and 0.1% Triton X-100. Applied TNF-α gene sequences were synthesized by 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. HWE in the controls was tested by comparing the expected and observed genotype frequencies using Pearson’s Chi-squared. The analysis was performed to
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[3].

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

Table 2: TNF-α polymorphisms in patients with AMD compared to the controls

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

Table 3: Association of TNF-α complex genotypes with AMD

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

TNF-α: Tumor necrosis factor-α; AMD: Age-related macular degeneration.
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, -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[16]. 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-α 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 molecular mechanism of obtained results is not clear. Using genotypes and haplotypes analysis the association of certain molecular mechanism of obtained results is not clear. Using genotypes and haplotypes analysis the association of certain molecular mechanism of obtained results is not clear. Using genotypes and haplotypes analysis the association of certain molecular mechanism of obtained results is not clear. Using genotypes and haplotypes analysis the association of certain molecular mechanism of obtained results is not clear. Using genotypes and haplotypes analysis the association of certain molecular mechanism of obtained results is not clear. Using genotypes and haplotypes analysis the association of certain molecular mechanism of obtained results is not clear. Using genotypes and haplotypes analysis the association of certain molecular mechanism of obtained results is not clear. Using genotypes and haplotypes analysis the association of certain molecular mechanism of obtained results is not clear.

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


