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CLINICAL STUDY

TNF-α promoter polymorphisms and primary open-angle glaucoma

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Abstract

Purpose **Primary open-angle glaucoma (POAG) is a multifactorial optic neuropathy with a strong hereditary component. Recent studies suggested a role for tumour necrosis factor-α (TNF-α) in the pathogenesis of POAG. The purpose of the present study was to investigate a hypothesized association between the TNF-α −308G > A and −238G > A gene polymorphisms and the presence of POAG in a Caucasian population.**

Methods **The present case–control study comprised 114 unrelated patients with POAG and 228 healthy control subjects, matched for age and gender. Genotyping of the TNF-α −308G > A and −238G > A polymorphisms was performed using polymerase chain reaction.**

Results **Allelic frequencies and genotype distributions of both the TNF-α −308G > A and −238G > A gene polymorphisms did not significantly differ between patients with POAG and control subjects. Presence of the TNF-α −308A-allele was associated with an odds ratio (OR) of 0.96 for POAG, whereas an OR of 0.52 was found among carriers of the TNF-α −238A-allele.**

Conclusion **Our data suggest that none of the investigated TNF-α gene polymorphisms is a major risk factor among Caucasian patients with POAG.**

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Keywords: open-angle glaucoma; tumour necrosis factor-α; genetic polymorphism

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Introduction

Primary open-angle glaucoma (POAG) is one of the major causes of blindness throughout the world.[1] It is defined as a multifactorial optic neuropathy with apoptotic retinal cell death leading to cupping of the optic nerve with typical visual field defects.[2] Population-based studies on the genetic influence have shown a high heritability of POAG.[3,4] Elevated intraocular pressure is the most important and so far only modifiable risk factor. However, there is still a number of patients with disease progression despite aggressive pressure-lowering therapy.[5] Consequently, previous studies focused on vascular, immunologic, and neurotoxic factors, which have subsequently been shown to contribute to the pathogenesis of POAG.[6–8]

In recent years, tumour necrosis factor-α (TNF-α), a major immunomodulator and proinflammatory cytokine, has been suggested to participate in the apoptotic death of retinal ganglion cells in glaucoma patients. Tezel *et al*[10] and Tezel and Wax[11] reported an upregulation of TNF-α and TNF-α receptor-1 in optic nerve heads and retina sections of glaucomatous eyes.[9,10] Furthermore, a cell culture study has shown that glial cells exposed to elevated hydrostatic pressure or stimulated ischaemia secreted increased amounts of TNF-α, subsequently leading to apoptotic death of cocultered retinal ganglion cells. This effect was attenuated by neutralizing antibodies against TNF-α.[11]

Over the last few years, several polymorphisms in the promotor region of TNF-α have been identified.[12] One of the most intensively studied polymorphism is characterized by a G to A substitution at position −308 (TNF-α −308G > A). Lipopolysaccharide (LPS)-stimulated whole blood cell cultures as well as peripheral blood mononuclear cells stimulated with anti-CD3 and anti-CD28 monoclonal antibodies from subjects carrying the TNF-α −308GA genotype showed a significant increase in TNF-α production compared to individuals carrying the TNF-α −308GG genotype.[13,14] Other studies, however, were unable to confirm this effect on TNF-α synthesis.[15–17] Another TNF-α polymorphism is characterized by a G to A

substitution at position −238 (TNF-α −238G > A). Huizinga *et al*[15] observed an increase in TNF-α production in LPS-stimulated whole-blood cell cultures among individuals carrying the TNF-α −238GG genotype, while in a study by Pociot *et al*[17] this genotype had no influence on TNF-α production.

Recently, Lin *et al*[18] reported an association between the TNF-α −308G > A gene polymorphism and POAG in a Chinese population. To the best of our knowledge, the present study is the first to investigate the role of the TNF-α −308G > A and the TNF-α −238G > A gene polymorphisms in Caucasian patients with POAG.

Materials and methods

The present case–control study comprised 114 unrelated patients with POAG and 228 unrelated control subjects. All participants were Caucasians from southern Austria and were seen at the Department of Ophthalmology, Medical University Graz between November 2002 and April 2004. Informed consent was obtained from all subjects prior to enrolment. The study was conducted in accordance with the standards of the local Ethics Committee and the National Gene Technology Act.

All patients underwent slit-lamp biomicroscopy, testing for best-corrected visual acuity, Goldmann applanation tonometry, gonioscopy, pachymetry, and standard automated perimetry (Interzeag Octopus 101, programme G2) or—in cases of profoundly decreased visual acuity—Goldmann perimetry. In all patients, photographs of the optic disc were taken. POAG was defined by an intraocular pressure before initiation of a pressure-lowering therapy of at least 21 mmHg, an open anterior chamber angle, optic disk changes characteristic for glaucoma (notching, thinning of the neuroretinal rim, increased cup/disc ratio in relation to the optic disc size), visual field defects characteristic for glaucoma (inferior or superior arcuate scotoma, nasal step, paracentral scotoma), and absence of conditions leading to secondary glaucoma.

The control group consisted of 228 unrelated patients with no morphological or functional damage indicative for primary or secondary open-angle or angle-closure glaucoma. Control subjects were admitted to our department for cataract surgery, and were matched to cases by gender and age (±2 years). Medical history concerning arterial hypertension, diabetes mellitus, cardiovascular events, smoking habits, and recent medication was obtained from all participants.

Analysis of genomic TNF-α polymorphisms

Venous blood was collected in 5 ml EDTA tubes. DNA was isolated using the QIAamp DNA blood mini-kit (QIAGEN, Netherlands) and stored at −20°C. All polymerase chain reactions (PCR) were run under conditions previously described.[19] Primer sequences for the gene polymorphism at −308 were forward 5′-GGG ACACACAAGCATCAAGG-3′ and reverse 5′-GGGACA CACAAGCATCAAGG-3′, and for the polymorphism at −238 forward 5′-ATCTGGAGGAAGCGGTA GTG-3′ and reverse 5′-AGAAGACCCCCCTCGG AACC-3′. DNA samples were amplified in 25 μl aliquots containing 200 μM deoxynucleoside triphosphate, 10 μM of each primer, 1.5 mM $MgCl_2$, 2 μl DNA sample, and 2 U *Taq* polymerase (Applied Biosytems, CA, USA). Annealing temperature was 62°C. The PCR products were digested at 37°C with *Nco*I to detect the single-nucleotide polymorphism in the −308 gene allele, using *Msp*I to detect the polymorphism of the −238 nucleotide. The PCR product was then subjected to 3% agarose-gel electrophoresis. 'No target' controls were included in each PCR batch to ensure that reagents had not been contaminated.

Statistical analysis

Statistical analysis was performed using SPSS 11.0 for windows. Metric values were analysed by Student's *t*-test. Proportions of groups were compared by χ^2 test. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated by logistic regression. The criterion for statistical significance was $P \leq 0.05$.

Results

In total, 114 patients (66 female and 48 male patients) with POAG and 228 control subjects (132 female and 96 male subjects), matched for age and gender, were enrolled. The mean age of patients was 72.3 ± 9.5 and 72.7 ± 9.6 years in control subjects. Baseline characteristics are shown in Table 1. Arterial hypertension and a history of stroke were found significantly more often in patients with POAG. Prevalences of diabetes mellitus, current smokers, and history of myocardial infarction were not significantly different between both groups.

No significant differences in either genotype distribution or allelic frequencies of both the TNF-α −308G > A and the TNF-α −238G > A polymorphisms were found between patients with POAG and control subjects (Table 2). Presence of the TNF-α −308A-allele was associated with an OR of 0.96 (95% CI: 0.6–1.54) for POAG, whereas an OR of 0.52 (95% CI: 0.21–1.29) was calculated for subjects carrying the TNF-α −238A-allele. The observed genotype distributions did not deviate from those predicted by the Hardy–Weinberg

Table 1 Baseline characteristics of patients and controls

	Patients with POAG (n = 114)	*Control subjects (n = 228)*
Mean age (±SD)	72.3±9.5	72.7±9.6
Range (years)	45.5–92.5	44.0–91.0
Arterial hypertension	65 (57.0)	103 (45.2)*
Diabetes mellitus	19 (16.7)	37 (16.2)
Current smoker	9 (7.9)	14 (6.1)
History of myocardial infarction	10 (8.8)	11 (4.8)
History of stroke	14 (12.3)	11 (4.8)*

Numbers are given as *n* (%); *$P < 0.05$.

Table 2 Distribution of TNF-α −238G > A and TNF-α −308G > A genotypes

	Patients with POAG (n = 114)	*Control subjects (n = 228)*
TNFα −238GG	107 (93.9%)	205 (89.9%)
−238GA	7 (6.1%)	23 (10.1%)
−238AA	—	—
TNFα −238A-allele frequency	0.031	0.050
TNFα −308GG	79 (69.3%)	161 (70.6%)
−308GA	35 (30.7%)	61 (26.8%)
−308AA	—	6 (2.6%)
TNFα −308A-allele frequency	0.154	0.160

Numbers for genotypes are *n* (%).

equilibrium, and for control subjects were similar to those reported for a Caucasian population.[12]

Discussion

In experimental models, increased TNF-α concentrations have been shown to participate in the apoptosis of retinal ganglion cells.[9–11] Gene polymorphisms leading to increased synthesis of TNF-α may thus contribute to the pathogenesis of POAG. Indeed, an increased prevalence of the TNF-α −308A-allele has recently been reported in Chinese patients with POAG.[18] The role of the TNF-α −308G > A polymorphism as a potential risk factor, however, has not yet been assessed in a Caucasian population. The present study is also the first to investigate a hypothesized role of the TNF-α −238G > A gene polymorphism in POAG.

Genotypes of the TNF-α −308G > A and the TNF-α −238G > A polymorphisms were determined in 114 patients with POAG and 228 control subjects, matched for age and gender. Allelic frequencies as well as genotype distributions did not significantly differ between both groups. An OR of 0.96 for POAG was found in carriers of the TNF-α −308A-allele, suggesting that this polymorphism is not a risk factor for POAG. This is in contrast to the findings of Lin *et al*,[20] who reported a significantly increased prevalence of the TNF-α −308A-allele among 60 Chinese patients with POAG with an OR of 2.72 for POAG among carriers of the TNF-α −308A-allele.[18] Possible explanations for these conflicting results may include small sample size as well as varying genotype distributions among different populations.

Interestingly, Funayama *et al*[20] investigated sequence variations in the optineurin gene, the expression of which is induced by TNF-α, and their association with polymorphisms in the promoter region of the TNF-α gene at positions −308, −857, and −863 in Japanese patients with POAG. With regard to TNF-α polymorphisms, no significant difference in genotype or allelic frequencies was found.

It is important to note that besides stimulation through LPSs, the production of TNF-α is induced by various other factors, such as free oxygen radicals and cytokines like interleukin-1 and γ-interferon.[21,22] Thus, our finding that the TNF-α −308G > A and the TNF-α −238G > A polymorphisms are not associated with an increased risk for POAG does not exclude a substantial role of TNF-α in the pathogenesis of POAG, but strongly suggests that none of the investigated TNF-α gene polymorphisms is a major risk factor among Caucasian patients with POAG.

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