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TNF-α Gene Polymorphisms and Primary Open Angle Glaucoma in Romanian Population

Polimorfisme ale genei TNF-α în populația română cu glaucom primar cu unghi deschis

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Abstract

Primary open-angle glaucoma (POAG) represents the most common form of a heterogeneous group of glaucomatous optic neuropathies which are a worldwide cause of irreversible blindness. Immune dysregulation and the genetic background are considered important risk factors. The influence on susceptibility to POAG of single nucleotide polymorphisms (SNPs) of tumor necrosis factor-α (TNF-α) was intensively studied, mostly on Asian population. We investigated the possible association of two TNF-α SNPs (-308G/A and -857C/T) with susceptibility to POAG and its clinical characteristics. A case-control association study of aforementioned TNF-α SNPs was performed on 197 POAG patients (divided into two subgroups: high-tension and normal-tension glaucoma - HTG/NTG) versus 208 ethnically matched controls. This is the first study performed on Romanian population. No significant differences were found in terms of allelic frequencies, genotype distribution of the studied SNPs, or their haplotypes between POAG and healthy control groups. In the subgroup analysis, TT genotype of TNF-α -857T-allele was found to be associated with higher values of central corneal thickness (CCT) in NTG subgroup (p-value 0.032). In order to confirm the association between -857C/T SNP of TNF-α and CCT in NTG subgroup of POAG patients, additional studies on different populations should be performed.

***Keywords**: TNF-α polymorphisms;primary open-angle glaucoma;central corneal thickness*

Rezumat

Glaucomul primar cu unghi deschis (GPUD) este una dintre cele mai frecvente forme de glaucom și este una dintre principalele cauze de orbire la nivel mondial. Dereglări ale sistemului imun precum și terenul genetic reprezintă factori de risc importanți. Influența polimorfismelor mononucleotidice (SNP) ale TNF-α asupra susceptibilității la GPUD a fost intens studiată, cu precădere pe populația asiatică. În studiul nostru am investigat

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posibila asociere a SNP din pozițiile -308G/A și -857C/T din promotorul genei TNF-α cu susceptibilitatea la GPUD și caracteristicile sale clinice. Studiul este de tip caz-control și a fost efectuat pe 197 de pacienți cu GPUD, împărțiți în două subgrupuri: glaucom cu hipertensiune intraoculară și glaucom cu tensiune intraoculară normală (HTG/NTG) comparați cu un lot martor de 208 subiecți clinic sănătoși. Acesta este primul studiu realizat pe subiecți români. Nu s-au observat diferențe semnificative statistic în frecvențele alelelor minore, în distribuția genotipurilor studiate sau a haplotipurilor construite între lotul de pacienți cu GPUD și lotul martor. La analiza subgrupurilor, s-a observat o asociere între genotipul TT al SNP TNF-α -857C/T cu valori mai crescute ale grosimii centrale a corneei în grupul pacienților cu glaucom normotensiv (p-value 0.032). Aceste rezultate necesită confirmarea prin studii efectuate pe loturi lărgite și în populații diferite.

***Cuvinte cheie**: polimorfisme genice TNF-α, glaucom primar cu unghi deschis, grosimea centrală a corneei*

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Introduction

Glaucoma describes a group of heterogeneous optic neuropathy diseases and represents a major cause of irreversible blindness worldwide [1]. All forms of glaucoma have in common optic nerve degeneration and loss of retinal ganglion cells (RGCs) and are clinically characterized by typical visual field defects [2].

In most populations, primary open-angle glaucoma (POAG) is the most common type of glaucoma. POAG is characterized by an open anterior chamber angle appearance by gonioscopy and evidence of typical glaucomatous optic nerve atrophy, as provided by the latest revised definition of POAG by the American Academy of Ophthalmology in 2010 [3]. The typical optic nerve damage in POAG is represented by optic disc or retinal nerve fiber layer structural abnormalities (thinning, notching, focal narrowing of the neural rim, increases in cupping of the optic disc, peripapillary abnormalities) and functional abnormalities such as typical visual field defects [3]. The elevated intraocular pressure (IOP) is considered one of the major risk factors [4], most POAG patients presenting the high-tension glaucoma (HTG) form of disease. However, there are also POAG patients that have an IOP within normal range, those being classified as having normal tension glaucoma (NTG), a form of disease considered by some authors as a different entity [5].

Even if several factors were evaluated as risk factors for glaucoma, including family history of glaucoma, the primary cause of glaucoma is still unknown. However, it is considered that genetic background represents a major risk factor for all kinds of glaucoma. For example, it has been shown that relatives of the POAG patients have a higher risk (22%) of developing glaucoma compared to the relatives of the normal controls (2–3%) [6]. Furthermore, numerous familial genetic studies have shown the contribution of genetic variation to the development of this disease [7].

Most glaucoma forms may exhibit a heritable susceptibility consistent with complex trait inheritance (typically adult-onset forms of the disease) [8]. Hence, given its pattern of inheritance, POAG, including NTG, is considered a genetically complex multigenic disease [8].

Experimental data shows that the pathogenic mechanisms of POAG are complex and dysregulation of the immune system might be of most importance. Therefore, cytokines, such as tumor necrosis factor-α (TNF-α), seem to play an important role in the pathogenesis of POAG [9, 10]. Growing evidence indicates that TNF-α contributes to the pathogenesis of POAG in several pathways, mostly by induction of apoptosis in RGCs and therefore optic nerve degeneration. Apoptosis may also be directly induced through TNF-α receptor 1 (TNFR1) or indirectly through ischemia or elevated IOP [9-11]. Furthermore, in addition to its direct effect on the RGCs' ax-

ons, TNF-α may as well induce the expression of NOS-2 in the astrocytes of the optic nerve head with cytotoxic effects on RGCs [12].

The relationship between TNF-α and POAG has been extensively investigated worldwide at gene level with variable results in different populations. Among the most studied single nucleotide polymorphisms (SNPs) of the TNF-α gene were those located in the promoter region at positions -308 (rs1800629) and -238 (rs361525), which appear to influence TNF-α gene expression as shown on SNPedia database [13]. Other SNPs of TNF-α gene investigated in relation with POAG are those located at positions -1031(rs1799964), -863(rs1800630), -857(rs1799724) or -850 (rs1799724), -646 (rs4248160), -376 (rs1800750) which seem to have functional roles [13]. Our aim in this study was to investigate the possible association of -308G/A and -857C/T TNF-α gene promoter polymorphisms with POAG in a Romanian group of subjects. To our knowledge, this is the first study of TNF-α SNPs in a Romanian group of POAG patients.

Material and methods

Subjects

From October 2011 to June 2014 we recruited 197 patients with POAG (M/F 90/107; mean age =62,5) from two Ophthalmology centers ("Oculus" Clinic and Emergency University Hospital Bucharest, Romania). All patients in this study received comprehensive ophthalmic examinations, including IOP, visual acuity, standard automated perimetry, gonioscopy, pachimetry, optic disc examination and imaging. Patients with ocular diseases concomitant with POAG were excluded from our study.

POAG was defined as meeting all the following criteria: exclusion of secondary causes (e.g., trauma, uveitis, steroid-induced glaucoma, or exfoliation glaucoma), Shaffer grade III or IV open iridocorneal angle on gonioscopy, characteristic optic disc damage clinically observed and by Heidelberg Retina Tomograph II (HRT II) or Optical Coherence Tomography (OCT) (Cirrus HD-OCT, Carl Zeiss Meditec, Dublin, CA) or typical visual field loss by standard automated perimetry with the Glaucoma Hemifield Test (Humphrey Field Analyzer; Carl Zeiss Meditec, Inc., Dublin, CA). IOP was determined by Goldmann applanation tonometry.

All POAG patients satisfied the classification criteria for diagnosis of POAG as indicated by the European Glaucoma Society Terminology and Guidelines for Glaucoma [14].

The demographic and clinical features of the study subjects were summarized in Table I.

In the POAG group visual field scores were assessed by standard automated perimetry SAP 24-2 SITA Standard program of the Humphrey Field Analyzer in the most affected eye. The patients were classified using Hodapp classification to assess the severity of disease, the data collected being mean deviation (MD) obtained in Glaucoma Hemifield Test (GHT), recorded in decibels (dB). The central corneal thickness was assessed by non-contact pachimetry (Topcon 80-CT) and recorded in millimeters (mm).

Additionally, the patients were classified into the two considered subtypes of the disease: high-tension glaucoma (HTG) and normal-tension glaucoma (NTG), using the non-treated highest IOP at diagnosis associated with characteristic visual field defects and glaucomatous cupping.

Every POAG patient underwent photographs of the optic nerve in association with HRT II or OCT of the optic nerve and C/D ratio was considered the vertical ratio between aria and cupping of the nerve.

Medical history of arterial hypertension or diabetes mellitus was obtained and the use of anti-glaucomatous medication was also recorded.

Table I. Demographic and clinical characteristics of POAG patients.

Patients clinical characteristics, n (%) unless stated otherwise	POAG patients (n=197)
Mean age (years ±SD); age range	62.4 ±11.63; 30-86
Sex (M/F)	90 (45.7)/ 107 (54.3)
HTG/NTG subtype	139 (70)/58 (30)
IOP (mm Hg), mean (±SD), range (mm Hg)	17 (±5.6); 8-35
CCT (mm), mean (±SD), range (mm)	0.54 ± 0.038; 0.413-0.637
C/D ratio, mean (±SD), range	0.677 ± 0.151; 0.2-1
SAP MD (dB), mean (±SD), range	-7.28 ± 8.84; -31.7-+1.71
Arterial Hypertension	102/197 (51.7)
Diabetes mellitus	29/ 197 (14.7)

Legend: IOP=intraocular pressure (millimeters Hg); CCT=central corneal thickness (mm); SAP MD=mean deviation of the Standard automated perimetry (deciBells); C/D= vertical cup/disc ratio; SNP- single nucleotide polymorphism; POAG- primary open-angle glaucoma (n=197); HTG- high-tension glaucoma (n=139); NTG- normal-tension glaucoma (n=58); CO- healthy controls (n=208)

We used as control group a cohort of healthy Romanian individuals, potential organ donors (*n* = 208), recruited from "Prof. Dr. C. T. Nicolau" National Institute of Blood Transfusion, Bucharest, Romania. The healthy control group was matched with the POAG patients study group for sex ratio and mean age and all the study subjects were of similar ethnic background. Subjects in control group had no history of ocular diseases, particularly POAG. The study protocol was approved by the local ethics committee. The study details were explained to all subjects enrolled in our study and an informed consent for genetic screening was obtained in accordance with the tenets of the Declaration of Helsinki.

Genotyping of TNF-α Promoter Polymorphisms

Venous blood was collected in 5ml EDTA tubes. Genomic Deoxyribonucleic acid (DNA) was extracted from 200 microliters of blood samples with a commercial kit (Qiamp Blood Kit; Qiagen, Hilden, Germany), according to the manufacturer's protocol. DNA was stored at -20°C. Quantification of extracted DNA was performed by Nanodrop spectrophotometer. Patients and controls were genotyped for two TNF-α gene promoter polymorphisms: −857C/T (rs 1799724) and −308G/A (rs 1800629) using TaqMan SNP Genotyping Assays (Applied Biosystems, USA) on a 7300 Real Time PCR System (Applied Biosystems, USA). Genotyping of both polymorphisms was performed by allelic discriminating TaqMan Real-Time PCR method, according to the manufacturer's protocol.

Statistical Analysis

Statistical analysis of the SNPs was performed using SNPStats and PLINK programs [15,16,17] which are free statistical analysis tool sets, designed to perform a range of basic and large-scale analysis for genome-wide association studies in a computationally efficient manner. The Hardy-Weinberg equilibrium was tested using the Chi-square test. The minor allele frequencies of each SNP between POAG patients (and their subtypes, HTG or NTG) and control subjects were compared using Fisher's exact test. Allelic frequencies were rechecked by odds ratio with 95% confidence intervals (CI). Odds ratio (OR) and 95% confidence interval (CI)

were calculated after adjustment by confounders variables using the logistic regression method. The level of linkage disequilibrium (LD) was investigated automatically by the SNPStat program by matrices with selected statistics (D, D', and Pearson's r), calculating the deviation (D) between the expected haplotype frequency (under the assumption of no association) and the observed frequency, as well as the correlation coefficient between alleles (r), (for details, see ref 16-tutorial). Haplotype frequencies were estimated using the standard Expectation Maximization (E-M) algorithm and tested by logistic regression method [15,16]. The most frequent haplotype was automatically selected as reference category and rare haplotypes were pooled together in a group. The analysis of haplotypes assumes an additive model. In the association analysis of constructed haplotypes and POAG or HTG/NTG subtypes or their clinical characteristics, the results are shown either as OR and 95% CI when logistic regression test was used or differences in means and 95% CI when linear regression test results was used [15,16]. The results were considered statistically significant when the probability of findings occurring by chance was less than 5% ($p \leq 0.05$).

Results

We have performed a case-control association study of TNF-α gene SNPs (-308 G/A (rs1800629), and -857 C/T, (rs1799724) in a group of 405 Romanian unrelated subjects of which 197 POAG patients divided into two subgroups (139 with HTG and 58 with NTG) versus 208 ethnically matched controls. Both considered SNPs in the TNF-α gene were successfully genotyped in all study subjects.

None of the two TNF-α polymorphisms showed any deviation from Hardy-Weinberg equilibrium in the investigated groups.

The results for genotypes and minor allele frequencies for both -308 G/A and -857 C/T polymorphisms for the total group of POAG patients and for their subgroups compared with control group are presented in table II.

No significant differences in either genotype distribution or allelic frequencies of both the TNF-α gene polymorphisms (-308G/A and -857C/T) were found between patients with POAG or their subtypes (HTG and NTG) and control subjects. Presence of the TNF-α -308A-allele was associated with an OR of 0.95 (95% CI: 0.23-3.8; p=0.94) for POAG subjects, whereas an OR of 1.39 (95%CI: 0.56-3.46; p=0.14) was calculated for POAG subjects carrying the TNF-α -857T-allele. In the subgroup analysis, the presence of TNF-α -308A-allele

Table II. Genotype counts and frequencies and the frequencies of minor alleles of -308G/A and -856C/T TNF-a gene polymorphisms

SNP	Sequence Change	Genotype counts (AA/AB/BB‡; %)				Minor allele	Minor allele frequency			
		POAG	HTG	NTG	CO		POAG	HTG	NTG	CO
Rs 1800629	-308G/A	155/38/4 (0.79/0.19/0.02)	110/25/4 (0.79/0.18/0.03)	45/13/0 (0.78/0.22/0)	161/43/4 (0.77/0.21/0.02	A	0.12	0.12	0.11	0.12
Rs 1799724	-857C/T	117/72/8 (0.59/0.37/0.04)	79/55/5 (0.57/0.4/0.03)	38/17/3 (0.66/0.29/0.05)	137/58/13 (0.66/0.28/0.06)	T	0.22	0.23	0.2	0.2

Legend: ‡- A represents the common allele and B represents the minor allele

was associated with an OR of 1.46 (in a 95% CI: 0.36-5.98; p=0.72) with HTG, respectively an OR of 0.0 (p=0.16) with NTG subjects. Regarding the carriers of TNF-α -857T-allele, an OR of 1.64 (95% CI: 1.04-2.61, p=0.057) was calculated for HTG subjects, respectively an OR of 0.83 (95% CI: 0.23-3.07; p=0.94) for NTG subjects. Even if the results related to the association of HTG subjects and the carriers of TNF-α -857T-allele showed a tendency of association (p=0.057), these results have not reached the significant statistical level, suggesting that none of the investigated SNPs can be considered as a risk factor for POAG or their subgroups in Romanian population.

Linkage disequilibrium (LD) analysis revealed that -308G/A and -857C/T TNF-α polymorphisms were in LD (D≠0, D'~1, r< 0) (see table III), so further on, we analyzed the potential association of the haplotypes obtained by the combination of the studied SNPs of TNF-α and POAG and its subgroups. Four haplotype combinations could be constructed between these two polymorphic loci of TNF-α but only three were observed in each group. We have found no association between haplotypes of the -308G/A and -857C/T polymorphisms and the presence of POAG or their subtypes in the studied group (see table IV). OR was calculated for each haplotype compared with all the other haplotypes. Global haplotype association p-value was obtained by logistic regression test and p≤0.05 was considered as statistically significant difference.

Furthermore, we analyzed the influence of the both TNF-α polymorphisms on POAG's clinical characteristics (visual field scores obtained by standard automated perimetry, vertical cup/disc ratio measured by optical coherence tomography, central corneal thickness measured by pachymetry) in our POAG subgroups (HTG/NTG). We found no significant association between -308G/A or -857C/T SNPs of the TNF-α gene and the scores obtained by standard automated perimetry (interaction p values were p=0.8 for -308G/A respectively, p=0.86 for -857C/T association) or vertical cup/disc ratio (interaction p values were p=0.33 for -308G/A respectively, p=0.61 for -857C/T association) in POAG subgroups.

Related to the central corneal thickness (CCT) of POAG group, we found no significant association of -308G/A TNF-α polymorphism with the studied subgroups (interaction p-value=0.31). However, in the analysis of the TNF-α -857T-allele distribution in POAG subgroups (HTG/NTG), we found that the NTG patients with genotype TT have shown higher values of the CCT, difference maintained after the correc-

Table III. Results of linkage disequilibrium statistics for -308G/A and -857C/T TNF-α polymorphisms for Romanian POAG patients and controls

D statistic		
TNF-α 308G/A	TNF-α 308G/A	TNF-α -857C/T
TNF-α -857C/T	.	**-0.0254**
D' statistic		
TNF-α 308G/A	TNF-α 308G/A	TNF-α -857C/T
TNF-α -857C/T	.	**0.9981**
r statistic		
TNF-α 308G/A	TNF-α 308G/A	TNF-α -857C/T
TNF-α -857C/T	.	**-0.1912**

Legend: D statistic, D' statistic, r-statistic (Pearson's test) see ref 15, 16

Table IV. Haplotype Analysis of TNF-α in Patients with POAG and HTG/NTG subgroups vs. Healthy Control Subjects

Haplotype	Haplotype Frequency		P	OR (95% CI)	Haplotype Frequency	P	OR (95% CI)
(rs1799724 -rs1800629)	Controls	POAG			HTG/NTG		
GC	0.6755	0.6598	NA	1	0.6475/0.6897	NA/NA	1/1
GT	0.2019	0.2234	0.49	0.89 (0.63 -1.24)	0.2338/0.1983	0.33 /0.88	1.20 (0.83 - 1.74)/ 0.96 (0.59 - 1.58)
CA	0.1226	0.1168	0.92	1.02 (0.67 - 1.56)	0.1187/0.1120	0.95/ 0.74	1.02 (0.64 - 1.61)/ 0.89 (0.46 - 1.73)
Total	1.000	1.000	**0.76**†		1.000/1.000	**0.62/0.94**†	

Legend: POAG- primary open-angle glaucoma patients (n=197); Controls -healthy controls (n=208); HTG-high-tension glaucoma patients (n=139); NTG-normal-tension glaucoma patients (n=58); OR- odds ratio; CI-confidence interval; NA- not applicable; †: Global haplotype association p-value: p ≤0.05

tion for sex and age was performed (interaction p-value =0.032- see table V).

Following haplotype analysis related to CCT in the POAG subgroups, we found a significant association between GT haplotype and CCT in NTG subjects (see table VI), which also remained significant after correction for age and sex was performed.

Discussions

TNF-α is one of the most studied molecules related to the POAG pathogenesis during p3ast years. TNF-α seems to play a significant role in POAG pathogenesis as shown by experimental models of induced intraocular hypertension in rats [18] and in vivo studies of POAG patients [11,19,20] or in vitro cultured RGC

Table V. Genotype distribution of TNF-α gene promoter polymorphisms (-308G/A and -857C/T) related to central corneal thickness (CCT) for POAG subtype groups

	Genotype	HTG patients (n)	CCT mean (s.e.)	Difference (95% CI)	NTG patients (n)	CCT mean (s.e.)	Difference (95% CI)	Interaction p-value:
Genotype -308 G/A	**G/G**	110	0.54 (0)	0.00	45	0.55 (0.01)	**0.01 (0.00 - 0.03)**	0.31
	G/A	25	0.53 (0.01)	0 (-0.02-0.01)	13	0.53(0.01)	0.00 (-0.03 - 0.02)	
	A/A	4	0.56 (0.02)	0.02 (-0.01-0.06)	0	---	---	
Genotype -857 C/T	**C/C**	79	0.54 (0)	0.00	38	0.54 (0)	-0.00 (-0.02 -0.01)	**0.032**
	C/T	55	0.53 (0.01)	-0.01 (-0.02 - 0.01)	17	0.56(0.01)	0.02 (-0.00 – 0.04)	
	T/T	5	0.53 (0.01)	-0.01 (-0.04 - 0.03)	3	0.59 (0.02)	**0.05 (0.00 – 0.09)**	

Legend: n= number of patients; HTG- high-tension glaucoma patients (n=139) NTG- normal tension glaucoma patients (n=58); CCT- central corneal thickness in millimeters; s.e.- standard error ; CI-confidence interval; statistic test: linear regression

Table VI. Haplotype distribution of TNF-α gene promoter polymorphisms (-308G/A and -857C/T) related to central corneal thickness (CCT) for POAG subtypes groups.

Haplotype	Frequency	HTG	NTG
		Difference (95% CI)	Difference (95% CI)
GC	0.6598	0	0.00 (-0.01 - 0.02)
AC	0.1168	0.00 (-0.01 - 0.01)	-0.01 (-0.03 - 0.01)
GT	**0.2234**	**-0.01 (-0.02 - 0.00)**	**0.02 (0.00 - 0.04)**
Interaction p-value: 0.013			

Legend: HTG- high-tension glaucoma patients (n=139) NTG- normal tension glaucoma patients (n=58); CI-confidence interval; statistic test: linear regression

[10]. It has been shown that TNF-α contributes to RGC apoptosis either directly through its receptor TNFR-1, which has high expression on the RGCs' of POAG patients [11], or indirectly, through the cytotoxic mechanisms of oxidative stress mediators, activated by its actions [10,20]. It has also been shown in a recent meta-analysis that POAG patients have higher levels of TNF-α in the aqueous humor than healthy controls, thus supporting the involvement of TNF-α in the development of POAG [21].

It has been observed that by experimentally reducing the serum levels of TNF-α in a mouse glaucoma model using TNF-α blocking agents or by deletion of the TNF-α gene, the apoptotic effect on RGC was reduced [18]. Also, the same study showed that intravitreal injections of TNF-α increased RGC apoptosis in normal mice.

The influence of -308G/A SNP on TNF-α's gene function is widely investigated, most of the authors considering that the presence of A allele in position -308 may increase the transcription activity, even by six to seven fold according to Agarwal *et al.* [22,23]. Nevertheless, there are authors considering that -308 G/A SNP may be functionally silent or have selectable effects only when there is linkage with selectable HLA alleles [24, 25]. Obviously, the -308G/A SNP is playing a regulatory role in the expression of the TNF-a gene, which makes it a likely candidate for playing a role in POAG susceptibility and severity.

Our results related to TNF-α-308A-allele showing that this SNP can not be considered a risk factor for POAG in Romanian patients (OR of 0.95 in a 95% CI: 0.23-3.8; p=0.94) are similar to those obtained by Mossböck *et al.* (OR of 0.96 in a 95% CI: 0.6–1.54) in a study performed on a Caucasian population [26]. However, there are studies that found a positive significant association between the AA genotype of -308G/A TNF-α SNP and the POAG patients in Turkish or Chinese populations [27,28,29]. It is worth mentioning that the findings of Lin *et al.* on a Chinese population related to -308G/A TNF-α SNP, which reported a higher A allele frequency in POAG patients versus controls (OR: 2.72 in a 95%CI: 1.66–4.45) [28] are in contrast with those of Fan *et al.*, that found a higher G allele frequency only in the HTG group compared to controls (OR: 1.89 in a 95%CI: 1.14-3.13), study also performed on a Chinese population [29].

Regarding the influence of TNF-α -857T-allele on POAG's susceptibility we found only two reports, both of them performed on Asian Populations [29,30], which found no significant association between the TNF-α -857C/T polymorphism and POAG. To our knowledge, the role of the TNF-α -857C/T polymorphism as a potential risk factor for POAG or their sub-

types, as well the possible association of this SNP with clinical characteristics of POAG has not yet been assessed in a Caucasian population. Funayama et al., in their study performed on Japanese patients with POAG [30], investigated the associations between variations in optineurin and TNF-α genes, including those located at positions -308G/A and -857C/T in the TNF-α gene. This study, despite the lack of association between the studied individual SNPs of TNF-α gene and POAG, revealed a significant association between a mutation in the optineurin gene (412A) in combination with TNF-α -857T-allele in POAG patients, compared to controls. Furthermore, the study found that patients with 412A mutation in the optineurin gene associated with the presence of TNF-α -857T-allele had visual field scores that were significantly worse than those with no optineurin mutation [30]. These conclusions suggest that, even if the -857T-allele in TNF-α gene is not a risk factor itself for POAG or its clinical characteristics, this SNP combined with other gene's mutations may influence the susceptibility and/or clinical characteristics of POAG.

Because of the large number of studies on -308G/A and -857C/T TNF-α gene polymorphisms in relation to POAG and their subgroups generated conflicting results in different populations, recently there were published two meta-analysis related to this subject. In one meta-analysis, Xin *et al.* concluded that TNF-α -308G/A polymorphism is significantly associated only with HTG subgroup of POAG (OR=1.660 in a 95%CI=1.033-2.667) and not with NTG (OR=1.005 in a 95%CI=0.321-3.689) or POAG (OR=1.379 in a 95% CI=0.877 -2.170) [21]. An earlier meta-analysis performed by Yu *et al.* found no associations between TNF-α -308G/A polymorphism and any form of POAG (OR=1.39 in a 95%CI= 0.78-3.59) in any population regardless of their origin [32], those results being consistent with our findings. A possible explanation for these variations from one study to another might be the differences in the genetic background of populations, for example, the frequency of AA genotype of TNF-α -308G/A polymorphism varies in Caucasian population from Central and Eastern Europe between 0.0% and 3.9% (in Czech, respectively Poland) [31] whereas in the Asian population AA genotype frequencies of 2.5%, respectively 6.8% in Chinese population were reported [28,29,]. The genotype frequencies of both SNPs (-308G/A and -857C/T) obtained in our control lot (0.02% AA, respectively 0.06% TT) were compared with data available in the www.allelefrequencies.net database and were found to be in range of those reported in Central and Eastern European Caucasian populations [31]. Also, the small size of the study groups could explain the differences between the results of various studies [26, 27, 28, 29].

Regarding the relationship between the TNF-α -857C/T polymorphism (alone or in combination with -308G/A) and different forms of POAG, Xin et al. showed that this association was investigated in only two studies, both on Asian populations [21] and both studies showed no association between the studied SNP and POAG, results consistent with those obtained in our study. To our knowledge, this is the first study which assesses the possible influence of TNF-α -857C/T on susceptibility to POAG, as well as its possible associations with POAG's clinical characteristics in a Caucasian population.

Regarding the constructed haplotypes of -308G/A and -857C/T TNF-α gene polymorphisms, our results showed no association between these haplotypes and POAG as well as its subtypes. These results are in contrast with those of Fan *et al.* that found one constructed haplotype (CA) significantly associated with HTG (p=0.015) [29].

Since the Ocular Hypertension Treatment Study in the early 2000s [33,34], corneal thickness was intensively studied regarding its influ-

ence on IOP or as an independent risk factor for POAG. It widely varies among different populations and ethnic groups, genetic studies by Toh *et al.* [35] or Freeman *et al.* [36] showing that central corneal thickness is one of the most heritable characteristics of the ocular structure. It appears to remain unchanged or slightly decreases over time [37]. Besides the known effect on IOP measurement, related to increased corneal thickness that might induce over-estimation of the IOP in POAG or POAG suspect subjects, the possibility of CCT involvement in glaucoma risk in a biological manner has been introduced. Lesk *et al.* found in their study [38] a relationship between thinner CCT and changes of the optic nerve head and ocular blood flow in POAG, indicating it as a factor of severity after reducing the IOP, thus suggesting the existence of other possible mechanisms involved. To our knowledge, this is the first study that takes into consideration the possible association of TNF-α SNPs and CCT in POAG subtypes HTG/NTG.

Conclusions

Our results have shown that neither of the TNF-α studied polymorphisms (-308G/A and -857C/T) nor their combined haplotypes do not represent a risk factor for POAG or their subgroups in Romanian population. We found that TNF-alpha gene promoter polymorphism -857C/T is associated with central corneal thickness (CCT) in NTG patients at individual level and in combined haplotype with -308G/A SNP. Even if our results related to TNF-α -857T-allele and CCT in NTG subgroup patients have shown a positive association, we consider that these results need to be confirmed on larger sample size studies and different populations.

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Conflicts of Interest

The authors declare no conflict of interest.

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