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Combined effect of carrying both CFH (rs1061170) and TGFβ1 (rs1800469) gene variants on the risks of various forms of age-related macular degeneration

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Background: *Age-related macular degeneration (AMD) is one of the most common disorders that can lead to total central vision loss after choroidal neovascularization or geographic atrophy (GA). Because the genetic component of the disease plays an important role in the pathogenesis, has an impact on the clinical presentation, and determines the response to treatment, studies on the genetic component of AMD are relevant for better understanding the molecular mechanisms underlying the pathogenesis.*

Purpose: *To investigate associations among TGFβ1 C509T (rs1800469) and CFH T1277C (rs1061170) polymorphisms, their gene-to-gene interactions and the risks of various forms of AMD.*

Material and Methods: *This was a case-control study. The case group included 61 patients with AMD. Of these, 31 were diagnosed with late dry AMD (GA), and 30, with wet AMD (neovascular AMD or nAMD). Patients with nAMD were divided into two subgroups of 14 patients with type 1 or occult subretinal neovascular membrane (SNM), the SNM1 subgroup and 16 patients with type 2 or classical SNM, the SNM2 subgroup. The control group was composed of 50 individuals with no eye disease and of an age distribution similar to that of the case group. Polymerase chain reaction (PCR) and restriction analysis of gene amplification products were performed to determine TGFβ1 rs1800469 and CFH rs1061170.*

Results: *We found a significant effect of TGFβ1 C509T (rs1800469) and CFH T1277C (rs1061170) gene variants on the risks of various forms of AMD. CFH 1277TT genotype was associated with decreased AMD risk, whereas 1277CC genotype, with increased AMD risk (first and foremost, increased GA risk) ($p < 0.05$). TGFβ1 509CC genotype was associated with increased risk, whereas TGFβ1 509TT genotype, with decreased risk of both GA and SNM2.*

Conclusion: *For the first time, a combined effect of gene variants of interest on the susceptibility to the development of AMD has been investigated, and synergism between these variants in increasing the risk of certain forms of the disease (e.g., GA) established. The results obtained create prerequisites for developing individualized prediction of risk and novel treatment strategies for the disease.*

Keywords:

age-related macular degeneration, geographic atrophy, TGFB1 rs1800469 variant, CFH rs1061170 variant

Introduction

Age-related macular degeneration (AMD) is a chronic progressive eye disorder characterized by lesions in the macular region of the retina and subsequent progressive loss of central vision. It is usually associated with age and is the major cause of visual impairment and blindness in those over 50. In recent decades, however, early signs of AMD have been seen in people younger than fifty [1]. Of note is that the disease is also a leading cause of vision loss in the developed countries.

In the meta-analysis of incidence of AMD in Europe [2], the pooled annual incidence of any late AMD was 1.4 per 1000 individuals. The prevalence of any AMD in Europe has been reported to be as high as 18.3% [3]. Approximately 67 million of people in the European Union are currently affected by any AMD, and, due to population ageing, this number is expected to increase [2].

Most visual function loss occurs in the late disease phase through abnormal choroidal neovascularization (exudative or wet AMD) or geographic atrophy (or late dry AMD).

The prevalence of AMD varies according to race, suggesting the existence of genetic differences among races [4]. The genetic component of the disease plays an important role in the pathogenesis, has an impact on the clinical presentation, and determines the response to treatment. This is why studies on the genetic component of AMD are relevant for better understanding the molecular mechanisms underlying the pathogenesis and the development of novel gene-based therapies for the treatment of AMD [5]. Thus, numerous studies (including

whole-genome studies) have confirmed the role of complement factor H (CFH) gene and transforming growth factor-beta 1 (TGFβ1) gene variants in the development of eye pathology in the particular populations [6-11]. The whole-genome studies, however, usually investigate the role of common and rare gene variants, but not the associations among gene variants in the risk of the disease.

The purpose of this study was to investigate associations among TGFβ1 C509T (rs1800469) and CFH T1277C (rs1061170) polymorphisms, their gene-to-gene interactions and the risks of various forms of AMD.

Material and Methods

This was a case-control study. The case group included 61 patients with AMD. Of these, 31 were diagnosed with late dry AMD (geographic atrophy or GA), and 30, with wet AMD (neovascular AMD or nAMD). Patients with nAMD were divided into two subgroups of 14 patients with type 1 or occult subretinal neovascular membrane (SNM), the SNM1 subgroup and 16 patients with type 2 or classical SNM, the SNM2 subgroup. The control group was composed of 50 individuals with no eye disease and of an age and gender distribution similar to that of the case group (Table 1). Study subjects underwent visual acuity assessment, ophthalmic biomicroscopy and swept-sourse optical coherence tomography (SS-OCT) with a high-definition Triton Plus® SS-OCT system (Topcon Corporation, Tokyo, Japan). TGFβ1 C509T (rs1800469) and CFH T1277C (rs1061170) variants were determined in patients.

This study followed the ethical standards stated in the Declaration of Helsinki and was approved by the Ethics Committee of Odesa National Medical University (Minutes of the Meeting held on October 5, 2018). Informed consent was obtained from all individuals enrolled in the study.

Deoxyribonucleic acid (DNA) was extracted from the buccal epithelial tissue stored in DNA/RNA Shield (Zymo Research, USA) using the Quick DNA MiniPrep Plus Kit (Zymo Research, USA). Polymerase chain reaction (PCR) and restriction analysis of gene amplification products were performed to determine TGFβ1 rs1800469 and CFH rs1061170 (Table 2).

PCR amplification was carried out in a thermocycler (Analytic Jena, Jena, Germany) using a DreamTaq Green PCR Master Mix (Thermo Scientific, Waltham, MA) kit and specific oligonucleotide primers (Metabion, Munich, Germany) (Table 2). The primer sequences and restriction fragments employed were as per previously published protocols [10, 12]. DNA fragment amplification products were hydrolyzed using specific restriction endonucleases (Thermo Scientific). Restriction analysis products were electrophoresed on a 3% agarose gel. The DNA bands were visualized with a UV transilluminator (Cleaver Scientific Ltd, multiSUB™ Midi, Rugby, UK) (Fig. 1).

SPSS v.27 software was used for statistical analysis. Descriptive statistics and Pearson chi-square tests were calculated to compare the groups on genotype frequencies and genotype combination frequencies. Odds ratio (OR) and 95% confidence interval (CI) values were calculated to assess associations between genotype (or genotype combination) with AMD risk.

Two different approaches were employed to examine gene-to-gene interactions. The first approach was implemented through the comparative analysis of genotype combinations between the groups. The second approach was implemented through a multifactor dimensionality

Table 1. Age and gender characteristics of study subjects

Characteristic		Controls (n=50)	AMD (n=61)	Patients with GA (n=31)	Patients with SNM1 (n=14)	Patients with SNM2 (n=16)
Age, years (M ± m)		72.2 ± 8.2	73.8 ± 8.9	74.5 ± 9.3	72.9 ± 6.8	73.2 ± 10.1
Gender	Men, n (%)	18 (36.0%)	24 (39.3%)	14 (45.2%)	4 (28.6%)	6 (37.5%)
	Women, n (%)	32 (64.0%)	37 (60.7%)	17 (54.8%)	10 (71.4%)	10 (62.5%)

Note: n, number of patients; GA, geographic atrophy; SNM1, type 1 subretinal neovascular membrane; SNM2, type 2 subretinal neovascular membrane

Table 2. Reactants and conditions for PCR and subsequent restriction analysis

Gene/ gene variant	Primer sequence 5′ - 3′	Amplicon (base pairs)	Restriction enzyme	Size of the restriction fragment (base pairs)
TGFB1 C509T	F: AGGGGGCAACAGGACACCTTA	123	BspTI (AflII)	509CC: 123 509CT: 18*, 105, 123 509TT: 18*, 105
	R: GTCGCAGGGTGTTGAGTGACAG			
CFH T1277C	F: TTTTTGGATGTTTATGCAATCTT	244	Hin1II (NlaIII)	1277TT: 244 1277TC: 79, 165, 244 1277CC: 79, 169
	R: ACTGTGGTCTGCGCTTTTG			

Note: *, fragments shorter than 30 base pairs (bp) are not visualized in agar gel

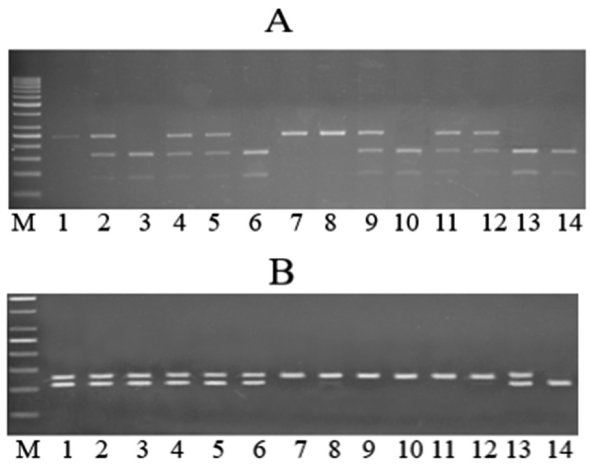

Fig. 1. Representing gel images showing (A) CFH T1277C polymorphism lanes: 1277TT genotype lanes (1, 7, 8), 1277TC genotype lanes (2, 4, 5, 9, 11, 12), 1277CC genotype lanes (3, 6, 10, 13, 14) and (B) TGFβ1 C509T polymorphism lanes: 509CT genotype lanes (1-6, 13), 509CC genotype lanes (7-12) and 509TT genotype lane (14), with 50–bnp DNA ladder represented by M.

reduction (MDR) method that had been proposed by Ritchie and employed in MDRv.3.0.2 software.

Permutation test (mdrptv.1.0 beta 2) was used to validate the models obtained. The level of significance $p \leq 0.05$ was assumed.

Results

Differences in frequencies of CFH and TGFβ1 gene variants between patients with AMD and controls were analyzed (Table 3). The CFH 509TT genotype was much more common in controls than in cases (46% versus 18%; Table 3), whereas CFH 1277CC genotype was much more common in cases than in controls (34.4% versus 10%), and these differences were significant. The differences found indicated that the homozygous 1277CC genotype was associated with an almost five times increased risk to develop AMD, whereas the homozygous 1277TT genotype was protective. In addition, there was no statistically significant association between the TGFβ1 gene variant and AMD risk, although 509CC genotype carriers tended to have greater but insignificant AMD risk.

At the next phase of the study, we compared CFH rs1061170 and TGFβ1 rs1800469 genotype frequencies between healthy controls and case groups (Table 4).

We found that the CFH 1277TT genotype was a protective genotype in the development of GA and SNM, because it was significantly less common in patients (16.1% and 20.0%, respectively) than in controls. It is noteworthy that there was no significant difference in the frequency of the homozygous 1277TT genotype between patients of the SNM1 subgroup and controls. Therefore, the protective effect of this genotype did not apply to the risk for developing occult SNM. However, the protective effect was seen in patients with SNM2, since the frequency of the genotype was 33.5% lower in the SNM2 subgroup than in controls, and this difference was significant.

Similarly, carriers of the homozygous 1277CC genotype showed a significantly increased risk of GA and SNM2, and there was no significant difference in the frequency of this genotype between patients with SNM1 and controls.

The TGFβ1 509CC genotype was found to be associated with an increased risk to develop GA, and was almost twice more common in patients with GA than in controls (51.6% versus 28%). There was, however, no difference in the distribution of other genotypes of the TGFβ1 C509T variant between patients with AMD and controls.

Of note is also a significantly increased frequency of the 509CC genotype ($\chi 2 = 4.06$, $p = 0.044$) and a significantly decreased frequency of the 509TT genotype ($\chi 2=3.97$, $p=0.046$) of TGFβ1 in patients with GA compared to those with SNM1 (Table 4).

We also assessed a combined effect of the gene variants of interest on various forms of AMD (Tables 5, 6). The protective effect of the CFH 1277TT variant was found to be neutralized in the presence of the 509CC homozygous variant (Table 5). In addition, the protective effect against AMD was found to be preserved in the presence of the TGFβ1 heterozygous genotype (-509CT) and CFH homozygous genotype (1277TT), which was indicated by a 16.5 increased frequency of AMD patients with this genotype compared to controls.

Table 3. Comparing CFH rs1061170 and TGFβ1 rs1800469 genotype frequencies between total patients with age-related macular degeneration (AMD) and healthy controls

Gene and variant		Controls (n=50)	AMD (n=61)	Results of statistical analysis
CFH T1277C	1277TT	23 (46.0%)	11 (18.0%)	χ^2=10.11, p=0.0015, OR=0.26 (0.11-0.61)
	1277TC	22 (44.0%)	29 (47.5%)	p>0.05
	1277CC	5 (10.0%)	21 (34.4%)	χ^2=4.73, p=0.0051, OR=4.73 (1.63-13.70)
TGFB1 C509T	509CC	14 (28.0%)	23 (37.7%)	p>0.05
	509CT	30 (60.0%)	32 (52.5%)	p>0.05
	509TT	6 (12.0%)	6 (9.8%)	p>0.05

Note: n, number of patients; χ2, Pearson's chi square coefficient; p, significance of difference; OR, odds ratio

A combination of the TGFβ1 homozygous genotype (509CC) with the CFH heterozygous genotype (1277TC) was associated with an increased risk for AMD, which was indicated by a 15.3% increased frequency of this combination in patients with AMD compared to controls.

Attention is drawn to that fact that no statistically significant difference was seen in the frequency of the combination of the TGFβ1 509CC and CFH 1277CC genotypes between patients with AMD and controls. This required detailed analysis, because, as we mentioned above, there was increased risk of various forms of AMD in carriers of these variants, and it might be expected that the negative effects of these genotypes on susceptibility to AMD would be summed. We subsequently found that carriers of the combination of the TGFβ1 509CC and CFH 1277CC genotypes had an increased AMD risk compared to non-carriers. This was indicated by the fact that, in the group of patients with AMD, the percentage of carriers of the combination was several times larger than in controls (Table 5).

We also examined the combined effect of the TGFβ1 and CFH gene variants on the risk for developing various

Table 4. Comparing CFH rs1061170 and TGFβ1 rs1800469 genotype frequencies between healthy controls and case groups (patients with graphic atrophy (GA), neovascular age-related macular degeneration (nAMD), type 1 subretinal neovascular membrane (SNM1) and type 2 subretinal neovascular membrane (SNM2))

Gene and variant		Controls (n=50)	GA (n=31)	nAMD (n=30)	SNM1 (n=14)	SNM2 (n=16)
CFH T1277C	1277TT	23 (46.0%)	5 (16.1%)[1]	6 (20.0%)[2]	4 (28.6%)	2 (12.5%)[3]
	1277TC	22 (44.0%)	13 (41.9%)	16 (53.3%)	8 (57.1%)	8 (50.0%)
	1277CC	5 (10.0%)	13 (41.9%)[4]	8 (26.7%)	2 (14.3%)	6 (37.5%)[5]
TGFB1 C509T	509CC	14 (28.0%)	16 (51.6%)[6]	7 (23.3%)	2 (14.3%)	5 (31.3%)
	509CT	30 (60.0%)	14 (45.2%)	18 (60.0%)	8 (57.1%)	10 (62.5%)
	509TT	6 (12.0%)	1 (3.2%)	5 (16.7%)	4 (28.6%)	1 (6.3%)

Comparing patients with a particular form of AMD with healthy controls:
[1] – χ^2=6.29, p=0.0122, OR=0.23 (0.07-0.68)
[2] – χ^2=4.77, p=0.029, OR=5.40 (1.37-21.26)
[3] – χ^2=4.44, p=0.035, OR=0.17 (0.03-0.82)
[4] – χ^2=9.52, p=0.002, OR=6.50 (2.02-20.89)
[5] – χ^2=4.77, p=0.029, OR=5.40 (1.37-21.26)
[6] – χ^2=4.58, p=0.032, OR=2.74 (1.08-7.00).

Note: n, number of patients; χ^2, Pearson's chi square coefficient; p, significance of difference; OR, odds ratio

Table 5. Comparing CFH rs1061170 and TGFβ1 rs1800469 genotype frequencies between total patients with age-related macular degeneration (AMD) and healthy controls

Gene and variant		Controls (n=50)	AMD (n=61)	Results of statistical analysis
CFH T1277C	TGFB1 C509T			
1277TT	509CC	14%	4.9%	p>0.05
	509CT	28%	11.5%	χ^2=3.87, p=0.0491, OR=0.33 (0.12-0.91)
	509TT	4%	1.6%	p>0,.05
1277TC	509CC	6%	21.3%	χ^2=4.05, p=0.0441, OR=4.24 (1.14-15.86)
	509CT	30%	21.3%	p>0.05
	509TT	8%	4.9%	p>0.05
1277CC	509CC	8%	11.5%	p>0.05
	509CT	2%	19.7%	χ^2=6.68, p=0.0098, OR=12.0 (1.50-95.87)
	509TT	0%	3.3%	p>0.05

Note: n, number of patients; χ2, Pearson's chi square coefficient; p, significance of difference; OR, odds ratio

Table 6. Comparing frequencies of combinations of CFH rs1061170 and TGFβ1 rs1800469 genotypes between healthy controls and case groups (patients with graphic atrophy (GA), neovascular age-related macular degeneration (nAMD), type 1 subretinal neovascular membrane (SNM1) and type 2 subretinal neovascular membrane (SNM2))

Gene and variant		Controls (n=50)	GA (n=31)	nAMD (n=30)	SNM1 (n=14)	CHM-II (n=16)	Results of statistical analysis
CFH T1277C	TGFB1 C509T						
1277TT	509CC	14%	9.7%	0.0%	0%	0.0%	p>0.05
	509CT	28%	6.5%[1]	16.7%	21.4%	12.5%	[1]χ^2=4.33, p=0.0375, OR=0.18 (0.04-0.84)
	509TT	4%	0.0%	3.3%	7.10%	0.0%	p>0.05
1277TC	509CC	6%	25.8%[2]	16.7%	14.3%	18.8%	[2]χ^2=4.28, p=0.0281, OR=5.45 (1.32-22.49)
	509CT	30%	16.1%	26.7%	21.4%	31.3%	p>0.05
	509TT	8%	0.0%	10.0%	21.4%	0.0%	p>0.05
1277CC	509CC	8%	16.1%	6.7%	0%	12.5%	p>0.05
	509CT	2%	22.6%[3]	16.7%[4]	14.3%	18.8%	[3]χ^2=6.94, p=0.0084, OR=14.29 (1.66-122.87) [4]χ^2=3.89, p=0.0485, OR=9.80 (1.09-88.48)
	509TT	0%	3.2%	3.3%	0%	6.3%	p>0.05

Note: n, number of patients; χ^2, Pearson's chi square coefficient; p, significance of difference; OR, odds ratio

forms of AMD. Significant differences were found only for the subgroup of patients with GA (Table 6). The protective effect from the presence of a genotype combination 1277TT/509CT (OR = 0.18) was not substantially different from that from the 1277TT only (OR = 0.23). The risk for the development of GA was, however, higher in carriers of this genotype combination than in carriers of one genotype only. In carriers of the combination of the CFH 1277CC genotype and the TGFβ1 heterozygous genotype (-509CT), the risk of developing GA (OR = 14.5) was increased compared to carriers of the former genotype only (OR = 6.5). In addition, the risk of developing GA was increased in carriers of the combination of the CFH heterozygous 1277TC genotype and the TGFβ1 genotype (509CC) was increased (OR = 5.45) compared to carriers of the latter genotype only (OR = 2.74).

Another aspect of the study was developing models of interaction of the gene variants of interest with such factors as age and sex in various forms of AMD. We used a multifactor dimensionality reduction (MDR) method to obtain such interaction models, but neither of them passed the permutation test, and, therefore, they were not statistically significant. In addition, we used MDR software to develop dendrograms for interaction of factors (particularly, gene-gene interaction, interaction of genes with other factors and interaction of other factors) in various forms of AMD (Fig. 2).

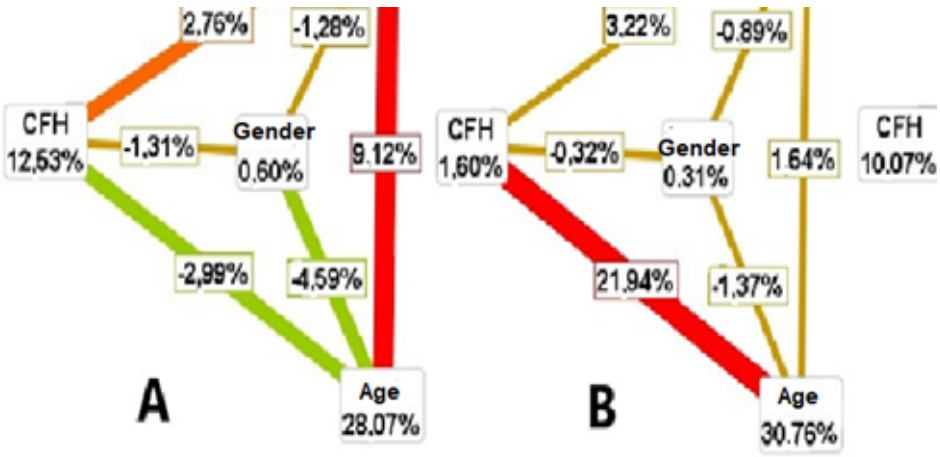

Fig. 2. Factor-factor interaction dendrograms. Red or orange lines indicate factors that may interact synergistically, whereas brown or green lines indicate factors that are redundant or do not interact. A. Assessing the risk for graphic atrophy. B. Assessing the risk for type 1 subretinal neovascular membrane. C. Assessing the risk for type 2 subretinal neovascular membrane.

Our analysis of graphic images found that most entropy in various AMD forms is associated with age. Among the three models (the model for the risk of GA, the model for the risk of SNM1, and the model for the risk of SNM2), the first-mentioned model showed the greatest gene-gene interaction entropy (2.76%) and the greatest percent of entropy explained by a single genetic factor (12.53% was attributed to the CFH T1277C polymorphism). With regard to various forms of AMD, the lowest percent of entropy explained by a single genetic factor was that attributed to gender.

Discussion

The etiopathogenesis of AMD has not been completely elucidated. Ophthalmologists consider AMD to be a multifactorial disease, with the major risk factors being age, gender, smoking, obesity, vitamin D deficiency, etc. Photoreceptor structure abnormalities developing with durable deformational alterations to the retinal pigment epithelium and neuroepithelium are believed to be a major cause of irreversible vision loss in AMD [13]. Finding early predictors of various forms of AMD could help clinicians in early therapy planning. Patient groups differing in phenotypic manifestations of AMD were found to differ in the mean magnitude of SS-OCT-derived subfoveal choroidal thickness [14]. The search for molecular and genetic predictors of the disease is an active and promising area of research. Anti-angiogenic therapy is a major treatment modality for a chronic retinal disease with choroidal neovascularization [15, 16, 17]. Patients with nAMD differ in response to treatment with antiangiogenic medications, which may be genetically determined. Numerous studies have demonstrated an important role of genetic factors in the development of the disease [18, 19]. Studies on the pathogenetic mechanisms of AMD are, however, commonly focused on particular genetic variants and do not take into account their interactions.

In the current study, we focused on the two molecular and genetic markers, TGFβ1 C509T and CFH T1277C polymorphisms, aiming to assess their genetic contribution to the risk of various forms of AMD in a cohort of patients from Ukraine, and investigate their gene-gene interaction in the development of the disease.

The glycoprotein CFH is a central regulator of the complement system, a major non-cell component of innate immunity involved in antimicrobial protection, immune complex processing and programmed cell death. CFH is synthesized in the liver and retina. It is involved in a mediated immune response and particularly inhibits an immune response and associated inflammation [20]. Under normal conditions, CFH binds to C-reactive protein (CRP) and prevents CTP-mediated proinflammatory responses [21] [21].

The CFH gene codes for complement factor H protein and is located on the long arm of chromosome 1 at 1q31.3. In a common polymorphism of the CFH gene (T1277C at rs1061170, or Y402H), thymine substitutes for cytosine at nucleotide 1277 in exon 9, with a resulting tyrosine-tohistidine change in amino-acid position 402 of the protein. The presence of this polymorphism results in less or no protection by CFH, leading to complement hyperactivation and, consequently, damage to cells, particularly, retinal cells [22]. It has been shown that carriers of the 1277CC genotype exhibited significantly reduced binding of CFH to CRP and malondialdehyde epitopes and derivatives present in the retina [23, 24]. Homozygous CFH Y402H carriers demonstrated higher levels of C5a, IL-18 and TNF-α in the Bruch's membrane and choroid [25]. The results of meta-analyses indicated that the strength of the association between the rs1061170 polymorphism and AMD varies among forms of AMD and ethnicities [26].

In the current study, we found that the frequency of 1277CC genotype was significantly increased in the entire group of patients with AMD, in patients with GA and in those with SNM2. Therefore, the CFH T1277C polymorphism is associated with the risk for AMD in the Ukrainian population as well as in white Europeans. This finding is important because it allows using the results of studies in European populations (e.g., on the impact of the CFH T1277C polymorphism on the efficacy of anti-VEGF therapy for exudative AMD). Thus, it has been shown that the CC genotype at CFH rs1061170 is associated with an unfavorable visual acuity course after bevacizumab or ranibizumab therapy [22, 27]. In addition, our findings will enable studies on the treatment of dry AMD (i.e., search for therapeutic options aimed at lowering the hyperactivation of the complement system).

Transforming growth factor-beta (TGFβ) is a class of secretory polypeptide signal molecules that is not only an auxiliary factor for connective tissue growth but also a major factor affecting epithelial cell regeneration and the hematopoietic system. TGFβ modulates cell proliferation, cell migration, matrix synthesis, wound contraction, calcification, and the immune response, and is involved in all major steps of tissue reconstruction, repair, and other functions [28]. It has three isoforms, TGFβ1, TGFβ2, and TGFβ3, of which TGFβ1 is the most active [29]. The TGFβ1 gene is located on chromosome 19 at q13.2. A TGFβ1 promoter single nucleotide polymorphism (SNP) C509CT (rs1800469) is the most common and best studied. Particularly, it has been linked to a nearly twofold difference in plasma TGFβ1 levels among individuals and with risk, progression, and outcome of numerous diseases [29]. There have been reports on the association of this SNP with primary open-angle glaucoma and high myopia [10, 11]. To the best of our knowledge, however, there have been no studies on TGFβ1 C509T gene polymorphism in patients with AMD.

In the current study, we demonstrated that TGFβ1 509CC polymorphism confers a risk for GA. It is quite reasonable since the development of GA may be considered as a short way to photoreceptor death without a phase of neovascularization. As mentioned above, TGFβ1 509CC is associated with low TGFβ1 levels. We, however, found

a significant difference in the frequency of the 509TT genotype between patients with GA and those with SNM1. That is, patients with the 509TT genotype had high TGFβ1 levels leading to the development of the membrane, i.e., wet AMD.

With regard to our analysis of gene-gene and factor-factor interactions, we found interaction between CFH and TGFβ1 gene variants while assessing the risk for GA. The gene variants of interest increase the effect of each other in GA (Tables 4 and 5), which was confirmed by the results of our factor-factor analysis. In addition, age was found to be the major factor in the development of various forms of AMD. The results obtained are in complete agreement with current data on risks for AMD [30]. Moreover, we found no substantial effect of gender on the development of AMD, although studies in some populations reported that female gender was a significant risk factor and women were affected more frequently than men [30].

Conclusion

First, we found a significant effect of TGFβ1 C509T (rs1800469) and CFH T1277C (rs1061170) gene variants on the risk for AMD. CFH 1277CC genotype was associated with increased AMD risk, whereas 1277TT genotype, with decreased AMD risk.

Second, TGFβ1 C509T (rs1800469) and CFH T1277C (rs1061170) gene variants differed in the relationship with the risks of various forms of AMD. TGFβ1 509CC genotype was a risk factor for GA. CFH 1277CC genotype was associated with increased risk of both GA and SNM2, whereas CFH 1277TT, with decreased risk of GA, nAMD and SNM2.

Finally, the risk for AMD was increased in the presence of the combined effect of carrying both variants of the genes of interest, but some combinations of variants were associated with decreased AMD risk. The protective effect of the CFH 1277TT variant was found to be neutralized in the presence of the homozygous TGFβ1 509CC. In addition, the protective effect against AMD was found to be preserved in the presence of the heterozygous TGFβ1 genotype (-509CT) and homozygous CFH genotype (1277TT). Moreover, the combined effect of carrying both TGFβ1 C allele and CFH C allele demonstrated further increased GA risk compared with non-carriers.

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