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LIPC rs10468017, *rs493258* and *LPL rs12678919* Role in Patients With Age-Related Macular Degeneration

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Abstract

Objective: Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly individuals in developed countries. The etiology and pathophysiology of AMD are not fully understood. Formation of drusen is the main pathological change in AMD. Lipids make up about 40% of drusen volume, thus possible relation between AMD and genes controlling lipid metabolism could provide novel insights into AMD. Our purpose was to determine the genotype frequencies of *LIPC rs10468017*, *rs493258* and *LPL rs126789919* in patients with AMD in Lithuanian population.

Methods: The study enrolled 279 patients with early AMD, 256 patients with exudative AMD, and 829 healthy controls (reference group). The genotyping was carried out using the RT-PCR.

Results: *LIPC rs10468017* polymorphism was associated with a decreased risk of early and exudative AMD, while *LPL rs126789919* polymorphism was associated with a decreased risk of exudative AMD and *LIPC rs493258* was only associated with a decreased risk of early AMD.

Conclusion: The study showed that *LIPC rs10468017, rs493258* and *LPL rs126789919* gene polymorphisms may have a protective role in AMD development.

Keywords: Age-related macular degeneration; LIPC rs10468017; LIPC rs493258; LPL rs126789919; Gene polymorphism; Early AMD; Exudative AMD; Lipid metabolism

Introduction

Age-related macular degeneration (AMD) is a degenerative disease affecting central part of the retina (macula). Half of the blindness cases in industrialized countries are AMD related [1]. This disease affects 2.5 million persons in Europe, and 1.75 million in the USA [2,3]. About 13.8% of population in Lithuania is also affected by AMD. AMD is a complex disease with many contributing factors: age, oxidative stress, inflammatory processes, genetic factors and others as well as their interrelationship [4].

Aging process causes changes in human retina such as the appearance of drusen, an ophthalmoscopically visible focal yellow deposition of acellular polymorphous debris between the retinal pigment epithelium and Bruch's membrane [5]. In early AMD, a large number (≤ 10) of drusen result in diffuse regions with hyperpigmentation or hypopigmentation [6,7]. Late form of AMD is classified into dry (geographic atrophy of the retinal pigment epithelium with the lack of neovascularization areas) and wet type (or exudative; new blood vessel formations in choroid, called the choroidal neovascularization areas, further leading to the formation of the disciform scars) [6]. The wet form of AMD causes more severe damage to the retina and more frequently leads to devastating consequences, such as vision loss, than the dry form of AMD [6,7].

In drusen lipids represent at least 40% of the volume [8]. Thus, lipid metabolism and particularly high-density lipoprotein may be involved in the pathogenic mechanism of AMD [8]. Lipid particles accumulate within Bruch's membrane prior to the development of basal deposits or drusen. This observation has led to the hypothesis that these lipid particles contribute to drusen formation during the development of AMD. It is thought that genetic predisposition accounts for 70% of the risk of the disease development [9]. Genetic variants in genes encoding components of lipid metabolism have been found to result in the deposit of lipid particles and the formation of drusen in the retina and BrM, thereby affecting retinal function [10]. For instance, connection of hepatic lipase (LIPC) and lipoprotein (LPL) genes to AMD was found by genome association studies and confirmed by several epidemiological studies [11,12].

LIPC gene encodes hepatic lipase, and is localized in the long arm of 15 chromosome, position 21-23 (15q21-q23). Liver cells express LIPC and excrete the enzyme into blood [13]. In the blood, the LIPC enzyme converts very low density lipoproteins (VLDL) and medium-density lipoproteins (MDL) to low density lipoproteins (LDL). Disruption of the LIPC function increases the risk of suffering from these diseases because the lack of the enzyme means that VLDL and MDL are not converted into LDL and HDL. As a result, cholesterol and triglycerides are not removed from the circulation [13].

LPL gene encodes lipoprotein and is localized on the short arm of chromosome 8, position 22 (8p22). This enzyme is found on the surface of capillary cells which play a key role in the digestion and

absorption of dietary fat. In the event of the mutation in LPL gene, permanent LPL enzyme deficiency may result in fat not being carried to the liver, heart and skeletal muscles. Decreased LPL enzyme activity leads to accumulation of the triglycerides in the circulation, hyperlipidemia, which increases atherosclerosis, heart attacks and the risk of AMD [14,15].

However, results of studies related to AMD in different cohorts on the reported HDL cholesterol metabolism genes, including the hepatic lipase (*LIPC*) and lipoprotein lipase (*LPL*) genes are inconsistent [12,16-19]. Some studies did not find associations between these genes and AMD [16,17], while other studies found associations with AMD [18].

Genotype-phenotype associations are known to vary from country to country (11-19), so the aim of our study was to determine the genotype frequency of *LIPC rs10468017*, *rs493258* and *LPL rs126789919* in patients with early and exudative AMD in Lithuanian subjects.

Ethics Statement

The study was approved by the Ethics Committee for Biomedical Research in Lithuanian University of Health Sciences (LUHS) (Number-BE-2-/13). All donors provided written informed consent in accordance with the Declaration of Helsinki. The study was conducted in the Department of Ophthalmology, Hospital of LUHS.

Methods

Study population

Study participants comprised of 279 patients with a diagnosis of early AMD, 256 patients with exudative AMD and 829 healthy controls.

Control group formation

The control group consisted of 829 subjects (530 women and 299 men, age range 19-91 years) who had no ophthalmologic pathology on examination and matched by gender distribution to the early and exudative AMD groups (Table 1). Since averages of age were significantly different between the groups, the age was included as confounding factor in further logistic regression analysis of genotyping results.

Characteristic	Group			P value
	Early AMD n=279	Exudative AMD n=256	Control n=829	
Men, n (%)	87 (31.2)	90 (35.2)	299 (36.1)	0.333*
Women, n (%)	192 (68.8)	166 (64.8)	530 (63.9)	
Age, average (SD)	76.56 (9.832)	71.59 (11.13)	61.82 (12.15)	<0.001**
*-not significant. **-significant				

Table 1: Demographic characteristics of the study population.

Ophthalmological evaluation

All study patients were evaluated by slit-lamp biomicroscopy to assess corneal and lenticular transparency. Classification and grading of lens opacities was performed according to the Lens Opacities Classification System III. During examination, intraocular pressure was measured. Pupils were dilated with tropicamide 1%, after which fundoscopy using a direct monocular ophthalmoscope and slit-lamp biomicroscopy with a double aspheric lens of +78 diopters was performed. Results of eye examinations were recorded on specially standardized forms. For detailed analysis of the macula, stereoscopic colour fundus photographs of the macula, centered at 45° and 30° to the fovea, were obtained with a Visucam NM Digital camera (Carl Zeiss Meditec AG, Germany).

The classification system of AMD formulated by the Age-Related Eye Disease Study (20) was used: early AMD consisted of a combination of multiple small drusen and several intermediate (63-124 μm in diameter) drusen, or retinal pigment epithelial abnormalities; intermediate AMD was characterized by the presence of extensive intermediate drusen and at least one large (≥ 125 μm in diameter) druse or geographic atrophy (GA) not involving the centre of the fovea; advanced AMD was characterized by GA involving the fovea and/or any of the features of neovascular AMD [20]. Early and exudative AMD was diagnosed by two ophthalmologists. Optical coherence tomography was performed on all AMD patients.

The following subject exclusion criteria were used: (i) unrelated eye disorders, e.g., high refractive error, cloudy cornea, lens opacity (nuclear, cortical, or posterior subcapsular cataract), except minor opacities, keratitis, acute or chronic uveitis, glaucoma, or diseases of the optic nerve; (ii) systemic illnesses, e.g., diabetes mellitus, malignant tumors, systemic connective tissue disorders, chronic infectious diseases, or conditions following organ or tissue transplantation; (iii) ungraded color fundus photographs resulting from obscuration of the ocular optic system or because of fundus photograph quality.

General-medical examination

Information on diabetes mellitus and systemic infectious and non-infectious diseases was obtained by a family doctor examination and data extract from medical documentation. All the patients were consulted by a general practitioner and a neurologist. All the patients completed a questionnaire about risk factors and clinical symptoms. Patients with no symptoms of typical chest angina and with no typical ischemic changes on the ECG were included into the final study group.

Only patients with early and exudative age-related macular degeneration and healthy controls without mentioned pathologies were included into our research.

DNA extraction and genotyping

DNA for the analysis of the *LIPC rs10468017*, *rs493258* and *LPL rs126789919* gene polymorphisms was extracted from venous blood white blood cells using a DNA purification kit based on the magnetic beads method (MagJET Genomic DNA Kit, Thermo Scientific, catalog number: K2721), according to the manufacturer's recommendations. DNA aliquots were stored at -20°C until analysis. The genotyping was carried out using the real-time polymerase chain reaction (RT-PCR) method. *LIPC rs10468017, rs493258* and *LPL rs126789919* single-nucleotide polymorphisms were determined using TaqMan® SNP Genotyping assays (Applied Biosystems) and their genotyping performed using a Rotor-Gene Q real-time PCR quantification system (Qiagen, USA). Thermal cycling conditions for PCR were, first, denaturing at 95°C for 10 min, followed by 45 cycles of 92°C for 15 s and 60°C for 1 min. 30 s. The Allelic Discrimination software (Qiagen, USA) was used to determine the individual genotypes, according to the fluorescence intensity rate of different detectors (VIC and FAM).

Statistical analysis

Statistical analysis was performed using the SPSS/W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA). The data are presented as absolute numbers with percentages in brackets, average values and standard deviations (SD). The frequencies of genotypes and alleles (in percentage) are presented in Table 2.

Hardy-Weinberg analysis was performed to compare the observed and expected heterozygous genotype frequencies of *LIPC rs10468017*, *rs493258* and *LPL rs126789919* polymorphisms using the χ^2 test in all groups. The distribution of the *LIPC rs10468017*, *rs493258* and *LPL rs126789919* single-nucleotide polymorphism (SNP) in the early and exudative AMD and control groups was compared using the χ^2 test or the Fisher exact test. Association of early and exudative AMD with genes polymorphisms was calculated by logistic regression analysis before and after controlling for age. Adjustment for age are presented as adjusted odds ratios (aOR) and its 95% confidence interval (95% CI) in Table 3.

Differences were considered statistically significant when $P<0.05$.

Results

Statistical analysis revealed that the genotypes distribution of the *rs10468017* polymorphism in early AMD and the genotypes distribution of the *rs12678919* polymorphism deviated from Hardy–Weinberg equilibrium at a significance level of 5% and *rs493258* polymorphism did not show significance deviation from Hardy-Weinberg equilibrium, as shown in Table 2.

Gene marker	Genotype/ Allele	Control n (%) (n=829)	P value HWE	Early AMD n (%) (n=279)	P value HWE	Exudative AMD n (%) (n=256)	P value HWE	P value
LIPC Rs10468017	CC	424 (51.1)	0.101	172 (61.6)	0.002	151 (59.0)	0.269	0.013
	CT	324 (39.1)		82 (29.4)		87 (34.0)		
	TT	81 (9.8)		25 (9.0)		18 (7.0)		
	C	1172 (70.7)		426 (76.3)		389 (76.0)		
	T	486 (29.3)		132 (23.7)		123 (24)		
LIPC rs493258	CC	295 (35.6)	0.267	112 (40.1)	0.28	101 (39.5)	0.161	0.079
	CT	386 (46.6)		136 (48.7)		111 (43.4)		
	TT	148 (17.9)		31 (11.1)		44 (17.2)		
	C	976 (58.9)		360 (64.5)		313 (61.1)		
	T	682 (41.1)		198 (35.5)		199 (38.9)		
LPL rs12678919	AA	720 (86.9)	<0.001	252 (90.3)	0.13	235 (91.8)	0.423	0.139
	AG	96 11.6)		25 (9.0)		20 (7.8)		
	GG	13 (1.6)		2 (0.7)		1 (0.4)		
	A	1536 (92.6)		529 (94.8)		490 (95.7)		
	G	122 (7.4)		29 (5.2)		22 (4.3)		

LIPC: Hepatic Lipase Gene; LPL: Lipoprotein Lipase Gene; AMD: Age Related Macular Degeneration; HWE: Hardy-Weinberg Equilibrium. P values <0.05 indicated in Bold are statistically significant.

Table 2: Frequency of *LIPC* (*rs10468017* and *rs493258*) and *LPL* (*rs12678919*) genotypes in the patients with early and exudative AMD and in the control group.

The genotypes distributions of *rs10468017* polymorphism in LIPC gene differed significantly between early AMD and exudative AMD patients and controls (P=0.013), We observed that genotype CC was statistically significantly more frequent in early AMD and exudative AMD compared to control group (61.6% *vs.* 51.1%; p=0.002 and 59.0% *vs.* 51.1%; p=0.028, respectively), while the frequency of genotype CT was only lower in early AMD compared to control group (29.4% *vs.* 39.1; p=0.004). The frequency of mutant T allele was significantly lower in early AMD and in exudative AMD patients than in controls (23.7% *vs.* 29.3%, P=0.01 and 24.0% *vs.* 29.3%, P=0.02, respectively).

The genotype distribution of the *rs493258* polymorphism in *LIPC* gene was significantly different between early AMD patients and controls as well (P=0.026). Statistical analysis revealed that TT genotype was less frequent in early AMD compared to controls (11.1% *vs.* 17.9%; p=0.008), and less frequent in early AMD compared to exudative AMD (11.1% *vs.* 17.2%; p=0.043). The frequency of mutant T allele was significantly lower in early AMD patients than in controls (35.5% *vs.* 41.1%, P=0.018).

Further analysisrevealed that genotype AA of the *rs12678919* polymorphism in *LPL* gene was statistically significantly more frequent in exudative AMD group than in control group (91.8% *vs.* 86.9%; p=0.033) while the mutant G allele was less frequently observed only in early AMD compared to control group (4.3% *vs.* 7.4%; p=0.015).

Binomial logistic regression models were conducted for all three examined polymorphisms (Table 3). The significant association was observed for *rs10468017* polymorphism with a decreased risk of early AMD under the codominant (OR=0.624; 95% CI: 0.462-0.842; P=0.002), dominant (OR=0.552; 95% CI: 0.354-0.860; P=0.002), overdominant (OR=0.649; 95% CI:0.484-0.870; P=0.004)) and additive (OR=0.764; 95% CI: 0.617-0.947; P=0.014) models (Table 3). In contrast, there were no statistically significant variables after adjusting for age (Table 3). Also, there was a decreased risk of exudative AMD under the dominant (OR=0.728; 95% CI: 0.548-0.967; P=0.028) and additive (OR=0.774; 95% CI: 0.619-0.967; P=0.024) models only before adjusting for age (Table 3).

Early AMD			
Polymorphisms		**OR; 95% CI; P**	**aOR; 95% CI; P ***
Codominant			
rs10468017	CT TT	0.624; 0.462-0.842; 0.002 0.761; 0.470-1.232; 0.267	0.771; 0.489-1.216; 0.263 0.500; 0.225-1.112; 0.089
rs493258	CT TT	0.928; 0.693-1.243; 0.617 0.552; 0.354-0.860; 0.009	1.181; 0.747-1.867; 0.477 0.435; 0.213-0.888; 0.022
rs12678919	AG GG	0.744; 0.468-1.182; 0.211 0.440; 0.099-1.961; 0.281	1.154; 0.584-2.280; 0.680 0.167; 0.015-1.824; 0.142
Dominant			
rs10468017	CT+TT	0.651; 0.494-0.859; 0.002	0.707; 0.461-1.085; 0.113
rs493258	CT+TT	0.824; 0.624-1.088; 0.172	0.949; 0.612-1.470; 0.814
rs12678919	AG+GG	0.708; 0.453-1.105; 0.128	0.958; 0.493-1.861; 0.899
Recessive			
rs10468017	TT	0.909; 0.568-1.455; 0.691	0.553; 0.253-1.205; 0.136
rs493258	TT	0.575; 0.380-0.870; 0.009	0.395; 0.203-0.766; 0.006
rs12678919	GG	0.453; 0.102-2.021; 0.299	0.165; 0.015-1.800; 0.140
Overdominant			
rs10468017	CT	0.649; 0.484-0.870; 0.004	0.847; 0.544-1.320; 0.463
rs493258	CT	1.091; 0.832-1.432; 0.527	1.486; 0.973-2.268; 0.067
rs12678919	AG	0.752; 0.473-1.194; 0.226	1.151; 0.596-2.319; 0.640

Additive			
rs10468017	T	0.764; 0.617-0.947; 0.014	0.733; 0.527-1.018; 0.064
rs493258	T	0.790; 0.648-0.963; 0.020	0.773; 0.568-1.052; 0.101
rs12678919	G	0.720; 0.485-1.068; 0.103	0.840; 0.470-1.500; 0.555
Exudative AMD			
Codominant			
rs10468017	CT TT	0.754; 0.558-1.019; 0.066 0.624; 0.362-1.074; 0.089	0.843; 0.501-1.419; 0.520 0.606; 0.254-1.448; 0.260
rs493258	CT TT	0.840; 0.617-1.144; 0.269 0.868; 0.579-1.302; 0.495	1.174; 0.690-1.998; 0.554 0.772; 0.394-1.515; 0.452
rs12678919	AG GG	0.638; 0.386-1.056; 0.081 0.236; 0.031-1.811; 0.165	0.980; 0.411-2.338; 0.963 0.261; 0.022-3.061; 0.285
Dominant			
rs10468017	CT+TT	0.728; 0.548-0.967; 0.028	0.785; 0.484-1.274; 0.327
rs493258	CT+TT	0.848; 0.636-1.131; 0.261	1.031; 0.632-1.683; 0.903
rs12678919	AG+GG	0.590; 0.362-0.963; 0.035	0.825; 0.362-1.880; 0.647
Recessive			
rs10468017	TT	0.698; 0.411-1.188; 0.185	0.644; 0.275-1.509; 0.311
rs493258	TT	0.955; 0.660-1.383; 0.807	0.710; 0.385-1.311; 0.274
rs12678919	GG	0.246; 0.032-1.891; 0.178	0.261; 0.022-3.064; 0.285
Overdominant			
rs10468017	CT	0.802; 0.598-1.076; 0.142	0.901; 0.542-1.497; 0.686
rs493258	CT	0.879; 0.662-1.165; 0.369	1.279; 0.788-2.075; 0.320
rs12678919	AG	0.647; .391-1.071; 0.090	0.997; 0.418-2.378; 0.995
Additive			
rs10468017	T	0.774; 0.619-0.967; 0.024	0.803; 0.557-1.157; 0.239
rs493258	T	0.914; 0.750-1.114; 0.373	0.920; 0.665-1.271; 0.612
rs12678919	A	0.600; 0.385-0.936; 0.024	0.768; 0.384-1.537; 0.456
*P was adjusted for age in logistic regression models. OR: odds ratio; 95% CI: 95% confidence interval.			

Table 3: Risk prediction of single examined polymorphisms in *LIPC* and *LPL* genes for early and exudative AMD development under the logistic regression models.

In addition for *LIPC rs493258* polymorphism, the decreased risk of early AMD was revealed under the codominant (OR=0.552; 95% CI: 0.354-0.860; P=0.009), recessive (OR=0.575; 95% CI: 0.380-0.870; P=0.009) and additive (OR=0.790; 95% CI: 0.648-0.963; P=0.020) models before adjusting for age, and under the codominant (OR=0.435; 95% CI: 0.213-0.888; P=0.022) and recessive (OR=0.395; 95% CI: 0.203-0.766; P=0.006) models after adjusting for age (Table 3).

The analysis of rs12678919 polymorphism in LPL revealed statistically significant variable under the additive model with 1.7-fold decreased risk of exudative AMD (OR=0.600; 95% CI: 0.385-0.936; P=0.024) (Table 3).

Discussion

We analyzed *LIPC rs10468017*, *rs493258* and *LPL rs126789919* polymorphisms in patients with early, and exudative AMD. Our results showed a protective role of these three gene polymorphisms: *LIPC rs10468017* polymorphism decreased risk of both forms of AMD (early and exudative), *LIPC rs493258* polymorphism decreased risk only of

early AMD, and *LPL rs12678919* gene polymorphism decreased risk of exudative AMD.

Regarding the results analyzing *LIPC* gene polymorphisms association with AMD, six studies found that LIPC gene can slow down AMD development [11,12,18,19,21,22]. Among these six studies, two studies analyzed *LIPC* (*rs10468017*) gene polymorphism [12,21] and three *LIPC* (*rs493258)* [11,18,22], while one study analyzed both gene polymorphisms [19]. Four studies found no association with AMD [16,17,23,24], two studies analyzed only *rs10468017* polymorphism association with AMD [16,17] and two other studies analyzed both *LIPC* gene polymorphisms [23,24]. Interestingly, there was one study [25] which found an association between *rs10468017* polymorphism in *LIPC* and a decreased risk of progression from large drusen to NV (HR=0.57, P=0.04), and from normal to intermediate drusen (HR=0.72, P=0.07) [25]. Also, there were two studies which have got opposite results and proved significant association between *LIPC* (*rs493258*) and AMD development [26,27]. It should be noted, that we are in agreement with these six studies [11,12,18,19,21,22], which found that LIPC gene polymorphisms (*rs10468017, rs493258*) may reduce possibility for AMD development. Reynolds et al. investigated 458 participants study, including 318 advanced AMD cases with either geographic atrophy (n=123) or neovascular AMD (n=195), and 140 controls [12]. Authors found that the minor T allele of the *LIPC* (*rs10468017*) gene was associated with a reduced risk of AMD [12]. Similar results were also obtained by Seddon et al. [21]. This study included (n=545) patients with advanced AMD and n=275 controls. The TT genotype of the *LIPC* (*rs10468017*) variant was associated with a reduced risk of AMD (p=0.014) for the TT genotype versus the CC genotype [21]. Similarly, Peter et al. evaluated 130 women with intermediate and late (both geographic atrophy and neovascular) forms of AMD and 1121 subjects without AMD. After multivariate analysis, a protective effect was detected among TT carriers compared with non-carriers for the HDL pathway gene, *LIPC rs493258*, for intermediate and late AMD (P=0.003) [18]. Another study, done by Yu et al. [11], goes in agreement with the previous studies. Scientists in this study investigated a total of 3066 subjects on the basis of ocular examinations and fundus photography and categorized them as control (n=221), intermediate drusen (n=814), large drusen (n=949), or advanced AMD (n=1082), and revealed protective role of T allele for intermediate drusen (p=0.045), large drusen (p=0.041) and advanced AMD ($P=1.8 \times 10^{-3}$) [11]. The fifth study, done by Merle et al. (Alienor study), analyzed *rs493258* and *rs10468017* (*LIPC*) in 963 elderly residents of Bordeaux, France. After multivariate adjustment, the TT genotype of the *LIPC rs493258* variant was significantly associated with a reduced risk for early and late (both geographic atrophy and neovascular forms) AMD (P=0.049 and P=0.03, respectively), but associations of *LIPC rs10468017* polymorphisms with AMD did not reach statistical significance [19]. This study goes in agreement with the study done by Neale et al. [26] analyzing 979 advanced AMD cases and 1,709 controls. Neale discovery data implicated the association between AMD and a LIPC in the HDL pathway (discovery P=4.53e-05 for *rs493258*) and *rs10468017*, (P=1.34e-08), as well; contrary to Merle et al. study, *LIPC rs10468017* gene polymorphism there did not reach statistical significance [19]. So, our study goes in agreement with all these six studies. But even four studies revealed opposite results and proved no association with AMD [16,17,23,24]. Fauser et al. investigated 1201 AMD patients (827 Dutch and 374 German) and 562 control subjects (476 Dutch and 86 German) of all AMD stages and found no association for variant in *LIPC* (*rs10468017*) and AMD [16]. No association was found with *LIPC rs10468017* and *rs493258* gene polymorphisms in Sobrin et al. study analyzing 749 patients with geographic atrophy and 3209 participants with choroidal neovascularization (CNV) [23]. Other study done by Zhang goes in agreement with the previous studies-this study group analyzed LIPC in 157 neovascular age-related macular degeneration (nAMD) patients, 250 polypoidal choroidal vasculopathy (PCV) patients and 204 controls without any macular abnormality. None of *LIPC (rs10468017, rs493258)* variants were significantly associated with nAMD, as well [24]. In agreement with this study goes Tian et al. study, which analyzed 535 AMD patients and 469 controls from 16 centers that spread from the north to the south of China. Of the 535 AMD patients 64 (12%) had early and 471 (88%) had advanced AMD. Among the patients with advanced AMD, 464 (98.5%) had neovascular and 7 (1.5%) had atrophic AMD. No association was found with *LIPL rs10468017* gene polymorphism and AMD [17]. On the other hand other four studies [16,17,23,24] deny results, and are in conflict with the previuos studies, which proved possible risk-reducing effect on AMD development [11,12,18,19,21,22]. One study revealed *rs10468017* in LIPC being associated with a decreased risk of progression from large drusen to NV [25], but it seems that this study can be in agreement with the previous six studies, which found that *LIPC rs10468017* can decrease AMD development.

Other two studies concerning the impact of *LIPC* (*rs10468017*, *rs493258*) on AMD are inconsistent, the results of these gene polymorphisms suggesting a possible significant role on AMD development [26,27]. Chen et al. executed a genome-wide association scan for AMD, and data revealed its strong association with *LIPC rs493258* gene polymorphism which increased risk of AMD (OR=1.14, 95% CI: 1.09-1.20), overall $P=1.3 \times 10^{-7}$, *P* follow-up=0.0012) [26]. Cipriani et al. carried out a genome-wide association study of AMD in the UK population with 893 cases of advanced AMD and 2199 controls and found LIPC rs493258 playing a significant role in AMD development with a decreased risk of AMD, (OR=0.89; 95% CI: 0.79-0.99; P=0.04) [27].

Another our analyzed gene was *LPL rs126789919*, and we also proved its protective role to exudative AMD development. Other studies analyzing LPL gene polymorphism association with AMD development showed different results. There are only six studies analyzing this gene polymorphism with AMD development. Four studies found no significant association with LPL and AMD development [16,17,22,24], two studies found statistically significant association between *rs1267819* and AMD development [19,26], and our results showed that *LPL rs12678919* gene polymorphism 1.7 times decreased the risk of exudative AMD, as we mentioned previously. Fauser with colleagues included all stages of AMD, and no significant associations were observed for variants in LPL (rs12678919) [16]. Another study done by Zhang tested 157 nAMD patients, 250 PCV patients and 204 controls without any macular abnormality, and *LPL* (*rs12678919*) gene polymorphism was not significantly associated with nAMD, as well [24]. Tian et al. in multicenter case-control study investigated 535 AMD patients and 469 controls in *LPL rs12678919*, and no association with all AMD forms was found in this study [17]. In agreement with these three studies goes Neale with colleagues, their study proved that *rs12678919* was not associated with AMD development (P=0.07) [22]. On the other hand, other two studies demonstrated that LPL may play a significant role in AMD development [19,26]. Merle et al. in Alienor study found the *LPL rs12678919* variant was associated with early AMD (OR=0.67, 95% CI: 0.45-1.00; p=0.05) [19], in agreement goes Chen et al. this

scientists group observed significant association between one of HDL-c-associated alleles near LPL (*rs12678919*) and AMD (OR=1.26; 95% CI:1.11-1.43; P=0.003 [26].

The strength of our study is a thorough clinical assessment of the patients. All the patients were consulted by a general practitioner and other sub-specialist. The patients with ischemic heart disease, cerebral ischemia, malignant tumors, rheumatoid diseases, and end-stage liver or renal diseases were excluded from the study as well.

The main limitation of this study is that patients with atrophic AMD were not included into our study. Quite a big number of patients were included into our research considering our population, but, looking to others studies for example consortiums, more people could have been included.

Patients with early AMD have to be followed up in order to find which form of AMD (wet or dry) will manifest in later years.

Conclusions

Our study demonstrated protective role of all investigated SNPs LIPC (*rs10468017* and *rs493258*) and LPL (*rs12678919*). LIPC *rs10468017* polymorphism was associated with a decreased risk of both forms of AMD (early and exudative), LIPC *rs493258* polymorphism was only associated with a decreased risk of early AMD, and *rs12678919* polymorphism in LPL gene was associated with a decreased risk of exudative AMD. However, further studies with bigger sample size are required to evaluate these associations not only in early and exudative forms of AMD but in atrophic AMD, as well.

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