Association of high-density lipoprotein and apolipoprotein E genetic variants with age-related macular degeneration

Associação de lipoproteína de alta densidade e variantes genéticas da apolipoproteína E com degeneração macular relacionada à idade

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ABSTRACT

Purpose: This study aimed to evaluate the association of age-related macular degeneration (AMD) with apolipoprotein E (APOE) variants and serum lipid profiles, including levels and fractions of total serum cholesterol (TC), low-density lipoprotein cholesterol (LDLc), and high-density lipoprotein cholesterol (HDLc), and triglycerides (TG).

Methods: Genotyping of APOE-*Hhal* was performed in 134 patients (study group, SG) and 164 individuals without AMD (control group, CG), aged 50-89 years. Lipid profiles were analyzed in a subgroup of 30 subjects of both groups, matched according to age and sex. The significance level was set at P<0.05.

Results: APOE E3/E3 was more prevalent (SG=74.6%; CG=77.4%), with no difference between both groups (P=0.667). The same result was observed for risk genotypes (APOE E -/2: SG=7.4%; CG=10.3%, P=0.624). Serum levels of TC, LDLc, and TG revealed similar median values between SG (193.5, 116, and 155 mg/dL, respectively) and CG (207.5, 120, and 123.5 mg/dL, respectively; P >0.05). For HDLc, a higher median value was observed in SG (53.3 mg/dL) versus CG (42.5 mg/dL; P=0.016). Logistic regression analysis showed the same value, and the HDLc/TC ratio was -11.423 (P=0.014), as also confirmed by an increase in HDLc in SG. The association between lipid profiles and apolipoprotein E genotypes was similar in both groups (P>0.05).

Conclusions: APOE-*Hhal* is not associated with AMD. However, an increase in serum HDLc level appears to exert a protective effect against the disease, irrespective of the genetic variants of apoE.

Keywords: Polymorphism; genetic; Apolipoprotein E; Triglycerides; Lipids; Cholesterol; Macular degeneration; Cholesterol, HDL; LDL-receptor related proteins

RESUMO

Objetivo: *Este estudo teve como objetivo avaliar a associação de degeneração macular relacionada à idade (DMRI) com variantes de alipoproteína E (APOE) e perfil lipídico sérico, incluindo níveis séricos de colesterol total (TC) e frações de proteínas relacionadas a receptor de LDL (LDLc) e HDL colesterol (HDLc), e triglicérides (TG).*

Métodos: *Realizou-se genotipagem de APOE-*Hhal *em 134 pacientes (grupo de estudo - SG) e 164 indivíduos sem a doença (grupo controle - CG), na faixa etária entre 50-89 anos. O perfil lipídico sérico foi analisado em um subgrupo de 30 indivíduos de ambos os grupos, pareados por idade e sexo. Admitiu-se nível de significância para valor-P<0,05.*

Resultados: *APOE E3/E3 prevaleceu (SG=74,6%; CG=77,4%), sem diferença entre os grupos (P=0,667), o mesmo ocorreu para genótipos de risco (APOE -/E2: SG=7,4%; CG=10,3%, P=0,624). Níveis séricos de TC, LDLc e TG mostraram medianas semelhantes entre SG (193,5; 116; 155 mg/dL, respectivamente) e CG (207,5; 120; 123,5 mg/dL respectivamente; P>0,05). Para HDLc notou-se valor de mediana elevado em SG (53,3 mg/dL) versus CG (42,5 mg/dL; P=0,016), constatado também na análise de regressão logística, cuja razão HDLc/TC mostrou coeficiente -11,423 (P=0,014), confirmando acréscimo de HDLc em SG. A relação entre perfil lipídico sérico e genótipos de APOE mostrou semelhança entre os grupos (P>0,05).*

Conclusão: *APOE-*Hhal *não se associa a DMRI, no entanto, o acréscimo no nível sérico de HDLc parece ter efeito protetor contra a doença, independente de variantes genéticas da apoE.*

Descritores: *Polimorfismo genético; Apolipoproteins E; Lipídeos; Triglicerídeos; Colesterol; Degeneração macular; HDL-colesterol; Proteínas relacionadas a receptor de LDL*

INTRODUCTION

Age-related macular degeneration (AMD) is an irreversible, progressive retinal disease as well as one of the major causes of blindness in individuals aged >55 years[1]. It can be classified as early, intermediate, or late, according to the presence of anomalies of the retinal pigmented epithelium (RPE), size of drusen (yellow pigments comprising cholesterol and apolipoproteins)[2], area of atrophy of the RPE (geographic atrophy), and presence or absence of choroidal neovascularization (exudative and atrophic forms)[2-4].

In addition to genetic predisposition, which accounts for 70% of the risk of disease development[5], advanced age is also considered a risk factor, which results in the deposition of lipid particles and formation of drusen in the retina (Bruch's membrane, BM), thereby affecting retinal function[6].

Apolipoprotein E (APOE) acts in the metabolism of triglyceride-rich lipoproteins (TG)[7] and is also present in RPE and BM[4]. This glycoprotein is encoded by a gene represented by three alleles, apoE2, apoE3, and apoE4; apoE2 and apoE4 are associated with increased and decreased risk of AMD, respectively[7].

The carotenoids lutein and zeaxanthin are absorbed together with intestinal fat and transported to the retina by low- and high-density lipoproteins (LDL and HDL, respectively)[8]. These carotenoids act as

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antioxidants, limiting oxidative damage to the retina cells, exerting a protective effect against AMD[9]. Elucidating the association between serum lipid profiles and genetic variants of apoE may establish the underlying molecular mechanisms for the development and prevention of this disease. The present study aimed to evaluate the association of APOE-*Hhal* polymorphism with lipid profiles in patients with AMD.

METHODS

A total of 298 subjects were studied, including 134 patients (age, 51-89 years; mean, 72 ± 9.3 years; sex, 50% male) with AMD (study group, SG), and 164 subjects (age, 50-81 years; mean, 59 ± 7.2 years; sex, 57% male) under ophthalmological treatment for complaints other than AMD (control group, CG). All subjects were examined and followed up by an ophthalmologist, and a questionnaire with personal data and medical history was provided to them. Exclusion criteria included age of <50 years and/or diagnosis of diabetes mellitus.

DNA was extracted from whole blood collected in edetic acid (EDTA) using the salting-out method[10]. APOE polymorphic fragments were amplified by polymerase chain reaction (PCR) (Mastercycler, Eppendorf) using 0.5 µL of each deoxynucleotide (0.8 mM), 2.5 µL 10´ PCR buffer, 2.5 µL 10% dimethylsulfoxide, 2.5 µL of each primer (2.5 µM), 0.2 µL Taq polymerase (5 U/µL), 11 µL Milli Q water, and 2 µL diluted genomic DNA (0.2 µg). *APOE* (rs429358 and rs7412) polymorphisms were analyzed using the following primers: P1: 5'-ACAGAATTCGCCCCGGCCTGGTACAC-3' and P2: 5'-TAAGCTTGGCA CGGCTGTCCAAGGA-3'[11]. Genomic DNA (50 ng) amplification (ESCO Thermocycler) comprised 30 cycles (94ºC for 5 min, 94ºC for 30 s, 65ºC for 30 s, and 72º for 30 s), followed by a cycle of 10 min at 72°C for the final chain extension. Moreover, for the analysis of restriction fragment length polymorphism, the amplification product was digested with *Hhal*, followed by 5.0% agarose gel electrophoresis at 110 V for 2 h and 30 min, staining with GelRed® (Uniscience), and visualization of the DNA fragments under ultraviolet light[12].

For serum lipid profile analysis [total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc), and triglycerides (TG)], electronic medical records of the patients were assessed according to the standards of the V Brazilian Guidelines for Dyslipidemia and Prevention of Atherosclerosis[13].

Statistical analysis was performed using the Minitab and GraphPad statistical software. A descriptive analysis with mean, standard deviation, median, and percentage was used. Qualitative variables were tested with Fisher's exact or chi-square test. Genotype distribution analysis was performed with Hardy-Weinberg equilibrium testing. Further, for quantitative variables with Gaussian distributions, analysis of variance (ANOVA) was applied to compare three or more groups. For quantitative variables without Gaussian distribution, the Mann-Whitney test was used to compare the two groups. Logistic regression analysis was applied to estimate the probability of AMD occurrence considering the analyzed variables. The alpha error was set at 5% with a significance level of P<0.05.

This study was approved by the Ethics Research Committee of the Medical School of Sao Jose do Rio Preto (FAMERP). All subjects were informed about the study and confirmed their willingness to participate by signing an informed consent.

RESULTS

The distributions of genotypes and allele frequencies of APOE-*Hhal* are presented in table 1. The homozygous wild-type E3E3 genotype and allele E3 was prevalent in SG (74.6% and 0.87, respectively) and CG (77.4% and 0.88, respectively), with no significant difference between both groups (P=0.667 and 0.654, respectively). The same result was observed for genotypes with at least one E4 allele (E -/4; P=0.328) and one E2 (E -/2; P=0.624).

Table 2 shows similar results for serum median levels of TC, LDL cholesterol (LDLc), and TG between SG (193.5, 116, and 155 mg/dL, respectively) and CG (207.5, 120, and 123.5 mg/dL, respectively, P>0.05). For HDL cholesterol (HDLc), a higher median value was observed in SG (53.3 mg/dL) than in CG (42.5 mg/dL; P=0.016). This finding was confirmed by logistic regression analysis, providing an HDLc/TC ratio of -11.423 (P=0.014). The lipid profile revealed no association with the genotypes of APOE-*Hhal* (P>0.05; Table 3).

Table 1. Distribution of genotype and allele frequencies of APOE-*Hhal* polymorphism in individuals with age-related macular degeneration (SG, Study Group) and in controls (CG)

APOE-*Hhal* genotype	SG (N=134)		CG (N=164)		P value
	N	%	N	%	
E2E2	0	-	0	-	-
E2E3	10	7.50	16	9.80	0.623
E2E4	0	-	1	0.60	-
E3E3	100	74.60	127	77.40	0.667
E3E4	23	17.20	20	12.20	0.294
E4E4	1	0.70	0	-	-
Total	134	100.00	164	100.00	
E -/4	24	17.90	21	12.80	0.328
E -/2	10	7.40	17	10.30	0.624
Allele	**N**	**Abs. freq.**	**N**	**Abs. freq.**	
E2	10	0.40	17	0.50	0.694
E3	233	0.87	290	0.88	0.654
E4	25	0.19	21	0.60	0.239
Total	268	1.00	328	1.00	

Fisher's or chi-squared tests; N= total number of individuals; Abs. freq.= absolute frequency.

Table 2. Median, minimum, and maximum values for lipid profile in patients with age-related macular degeneration (SG, Study Group) and in controls (CG)

Lipid profile (mg/dL)	SG (N=30)	CG (N=30)	P value
TC			
Median	193.5	207.5	0.662
Minimum	144.0	107.0	
Maximum	327.0	313.0	
HDLc			
Median	42.5	53.3	0.016*
Minimum	24.0	22.0	
Maximum	70.0	80.0	
LDLc			
Median	115.0	120.0	0.882
Minimum	70.0	51.0	
Maximum	255.0	237.0	
TG			
Median	151.0	123.5	0.813
Minimum	46.0	54.0	
Maximum	561.0	333.0	

Mann-Whitney Test; TC= total cholesterol; HDLc= high-density lipoprotein cholesterol; LDLc= low-density lipoprotein cholesterol; TG= triglycerides; SD= standard deviation; N= number of individuals. *= statistically significant.

Table 3. Median, minimum and maximum values for lipid profile in patients with age-related macular degeneration (AMD) and without (controls) considering APOE-HhaI polymorphism

	AMD (N=30)			Controls (N=30)			
	E-/2 (a)	E3E3 (b)	E -/4 (c)	E-/2 (d)	E3E3 (e)	E-/4 (f)	
Lipid profile (mg/dL)	N=5	N=22	N=3	N=3	N=24	N=3	P-value
TC							
Median	207.0	193.0	219.0	233.0	212.0	152.0	0.121
Minimum	175.0	144.0	172.0	191.0	123.0	107.0	
Maximum	210.0	327.0	227.0	278.0	313.0	157.0	
HDLc							
Median	50.0	42.5	42.0	54.0	54.0	44.0	0.191
Minimum	30.0	24.0	36.0	51.0	22.0	40.0	
Maximum	68.0	63.0	70.0	55.0	80.0	53.0	
LDLc							
Median	114.0	117.5	140.0	126.0	123.0	83.0	0.234
Minimum	106.0	70.0	107.0	98.6	55.0	51.0	
Maximum	138.0	255.0	142.0	206.0	237.0	93.0	
TG							
Median	164.0	152.65	145.0	192.0	120.0	80.0	0.544
Minimum	95.0	46.0	75.0	85.0	55.0	54.0	
Maximum	295.0	561.0	184.0	227.0	333.0	127.0	

One-way ANOVA test; TC= total cholesterol; HDLc= high density lipoprotein cholesterol; LDLc= low density lipoprotein cholesterol; TG= triglycerides; SD= standard deviation; N= number of individuals.

DISCUSSION

In this study, similar to other studies[14,15], an association of APOE-*Hhal* polymorphism with AMD could not be confirmed, which is in contrast to another study[16]. The E3E3 genotype and E3 allele were prevalent in patients and controls, as observed in another study[7]. It has been demonstrated a higher frequency of allele E3, particularly in individuals without the disease[17]. In this study, the sample size played an important role, as did the diverse ethnic background of the different populations. The Brazilian population is largely composed of individuals of Amerindian, African, and European descent[18].

Meta-analysis have clearly shown the protective effect of the E4 allele, even in heterozygotes[19], which were not observed in this study. Although the E2 allele is considered a risk allele[16], no difference in risk was observed between the two groups. This finding is in accordance with that of other studies[14,15].

The protective nature of apoE4 can be explained by the lack of cysteine residues at positions 112 and 158, in contrast to apoE2 and apoE3. This lack prevents the formation of disulfide bridges with other proteins, as found in the binding between apoE3 and apoA-II in HDL[20]. The inability of apoE4 to form dimers with other proteins found in HDL facilitates the transport of lipoproteins in MB, considering the small particle size, preventing the formation of drusen[20,21]. This prevention could be maximized by the concomitant presence of apoE4 and high levels of HDL. Although our study has not confirmed an association between genetic variants of APOE, it has shown higher levels of HDL, particularly in CG, consistent with another study[22], thereby suggesting that increased HDLc is a protective factor against AMD. However, Chakravarthy et al.[23] did not observe this association, and other reports indicate higher risks of diseases accompanying high serum levels of HDLc[24].

HDL particles are responsible for the transport of the carotenoids lutein and zeaxanthin, which are present in dark green leafy vegetables, maize, squash, broccoli, peas, and egg yolk[25]. In addition to their antioxidant activity, these carotenoids hold great potential for improving macular pigment density[26] and, therefore, help filtering blue light. Thus, HDL is studied for its possible key role in protecting against AMD[27].

Changes in the metabolism of cholesterol, particularly of HDL, may influence drusen accumulation and consequently promote AMD development. This suggests that increasing levels of HDLc associated with a carotenoid-diet rich could interfere with disease progression, particularly in patients currently under treatment, thereby improving its prognosis. Thus, reduced levels of HDLc can increase the disease risk, as found in this study.

Cholesterol can be obtained from systemic sources or recycled in the retina, through circulation of blood lipids. Thus, the retinal rod cells are capable of using both lipids from liver and those recycled from RPE[28]. This dual capability can explain the association between increased serum cholesterol level and AMD, which was not observed in this study. Some authors agree with this finding[3,23]; however, other authors disagree[22,29]. The sample size, age, sex, and patients' lifestyle should be considered, while evaluating published results.

The present study assessed the association of lipid profile with APOE-*Hhal* polymorphism. ApoE plays a role in the metabolism of TG-rich lipoproteins (chylomicrons and very LDL) as well as in the association of this metabolism with HDL[3,30]. Importantly, apoE3 and particularly apoE4 reveal affinity with the membrane receptor apoB/E, whereas apoE2 is does not, which influences serum levels of TC, LDLc, and TG[19]. Thus, APOE-*Hhal* genotypes could contribute to changes in the levels of serum lipids and consequently cause greater deposition in RPE and form drusen[28].

Patients and controls showed similar distributions of CT, LDLc, HDLc, and TG values for all genotypes of APOE-*Hhal*. However, the small sample size is a limitation of this study. The study aimed to match both groups (patients and controls) according to sex and age in order to minimize confounding factors in the statistical analysis of the association of lipid profiles with genetic polymorphism. The findings should be confirmed in further studies with larger sample sizes and varied populations.

CONCLUSION

APOE-*Hhal* has no association with AMD. However, increased serum levels of HDLc appear to offer protection against the disease, irres-

pective of the genetic variants of apoE. This observation should be confirmed in larger sample sizes and patient subgroups in different populations.

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