

AGE-RELATED MACULAR DEGENERATION: ITS ASSOCIATION WITH THE $\epsilon 4$ ALLELE OF THE APOLIPOPROTEIN E GENE

DEGENERACIÓN MACULAR ASOCIADA A LA EDAD: ASOCIACIÓN CON EL ALELO $\epsilon 4$ DEL GEN DE LA APOLIPOPROTEÍNA E

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ABSTRACT

Objective: The apolipoprotein E (APOE) $\epsilon 4$ allele is a well-established factor associated with age-related macular degeneration (AMD). This disorder is characterized by a typical progressive central visual impairment. Our aim was to study further the relationship of the APOE genotype associated with AMD.

Materials and methods: We evaluated a group of 95 patients with AMD and 65 controls. The APOE genotype for each participant in the study was determined.

Results: The allele was associated with age-related macular degeneration (OR = 5.6; 95% CI= 3.4-8.8 ; $p < 0.001$). Female patients with at least one $\epsilon 4$ allele had a significantly earlier age at diagnosis ($\epsilon 4+$ = 72.2 S.D. 5.1; $\epsilon 4-$ = 78.8 \pm 5.7; $p < 0.001$).

Conclusions: The APOE $\epsilon 4$ allele is not a protective factor for AMD, but is associated with an increased risk of its development (*Arch Soc Esp Ophthalmol* 2006; 81: 9-12).

Key words: Apolipoprotein E, allele $\epsilon 4$, age-related macular degeneration.

RESUMEN

Objetivo: El alelo $\epsilon 4$ de la apolipoproteína E (APOE) es un factor de riesgo bien establecido en la degeneración macular asociada a la edad (DMAE). Esta enfermedad se caracteriza por pérdida progresiva de la visión central. Nuestro objetivo es estudiar la asociación del genotipo APOE con la DMAE.

Material y método: Se estudió un grupo de 95 pacientes con DMAE y 65 controles. A todos se les determinó el genotipo de la APOE.

Resultados: El alelo $\epsilon 4$ se asoció a degeneración macular asociada a la edad (OR = 5,6; 95% CI= 3,4-8,8 ; $p < 0,001$). La edad de diagnóstico es significativamente inferior en las pacientes que tienen al menos un alelo $\epsilon 4$ ($\epsilon 4+$ = 72,2 D.E. 5,1; $\epsilon 4-$ = 78,8 \pm 5,7; $p < 0,001$).

Conclusiones: El alelo $\epsilon 4$ de la APOE no es un factor de protección para la degeneración macular asociada a la edad y se asocia a un mayor riesgo de desarrollarla.

Palabras clave: Apolipoproteína E, alelo $\epsilon 4$, degeneración macular asociada a la edad.

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INTRODUCTION

Age-related macular degeneration (ADM) is the first cause of irreversible blindness in industrialised countries, with a prevalence of 13% in the 85-year age group (1). The ethiopathogeny of this disease is unknown although it is believed that ADM can be of genetic origin, even though environmental factors have a great influence (2). In recent decades, its pathology has been increasingly researched in order to determine its causes and risk factor, among which genetic factors are predominant (2-8).

The apolipoprotein E gen (APOE) is the first susceptibility gen related to AMD. APOE is a 299-aminoacid polymorphic plasmatic protein involved in the transport of cholesterol and other lipids, which has been demonstrated in druses and basal deposits (9). It is synthesised mainly in the liver, with the brain being the second synthesis organ. In the retinal pigmentary epithelium there is a regulation in the expression of APOE (10). It presents 3 isoforms: E2, E3, E4, coded by three alleles $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, with a single locus in chromosome 19 (11). In the general population the frequency of $\epsilon 2$ is of 5%, of allele $\epsilon 3$ 85% and of allele $\epsilon 4$ 10%. In different papers it has been established that carrying an allele $\epsilon 4$ is a protection factor for AMD (12-14). the APOE genotype could explain 50% of genetic variability of this disease. Other studies prove the lack of association between APOE and AMD in families (15).

The object of this study is to establish the relationship of genotype APOE and AMD in a sample of retina practice outpatients and study the association with APOE gen allele $\epsilon 4$.

SUBJECTS, MATERIAL AND METHODS

This study was approved by the Research and Training Committee of our hospital. Patients were recruited from the retina practice. 160 individuals were studied (95 patients and 65 controls) between 2002 and 2004. Table I shows the demographic characteristics of both groups. The inclusion criteria for patients were as follows:

- Accepts to participate in the study, signing informed consent.
- Early AMD (white druses(>63 μm) with or without pigmentation) or late AMD (neovascular or geographic form).

Table I. Demographic Data

	Control group	AMD group
N.º	65	95
Age	72.6 D.E. 6.8	75.6 D.E. 6.1
Sex	52.5% women	60.6% women
Diagnosis age		72,9±6,4

AMD: age-related macular degeneration; N.º: No.

- No association with other retinal dystrophy.
 - No family history of malattia léventinese.
- The inclusion criteria for controls were:
- Accepts to participate in the study, signing informed consent.
 - Being the spouse (no blood relation) of patients of the above group.
 - Not having AMD.

A complete ophthalmological exploration was made, including visual acuity, fundus study and fluorescein angiography. The APOE alleles were analysed with PCR. Blood samples were taken from participants on an empty stomach and stored in different proportions at -80°C , and leukocytes for extracting DNA. The enzymatic restriction products were prepared in small sized polyacrylamide gel. The allocation of genotypes ($\epsilon 2/\epsilon 2$, $\epsilon 2/\epsilon 3$, $\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 3$, $\epsilon 3/\epsilon 4$, $\epsilon 4/\epsilon 4$) was made by two independent individuals who were not aware of the diagnosis. To facilitate the study, patients were grouped on the basis of the presence or absence of APOE allele $\epsilon 4$: $\epsilon 4+$ group (having one or two $\epsilon 4$ alleles), and the $\epsilon 4-$ group (having none). The groups were divided in 5-year subgroups from 65 (the youngest patient) up to 80 (the oldest).

In order to compare the qualitative variables between patients and the control group the Chi square or Fischer test was used, with the t of Student for quantitative variables. The association between APOE gen allele $\epsilon 4$ and diagnosis was calculated by the odds ratio calculation (OR) and it's corresponding confidence ratio up to a confidence ratio of 95%. Utilizing logistical regression we assessed the risk depending of the APOE gen allele $\epsilon 4$, considering as a dependent variable or responde the presence or absence of AMD and, as independent variable or exposure factor, the number of alleles $\epsilon 4$. Utilizing multiple regression, we analyzed the diagnostic age of AMD and allele $\epsilon 4$. The results were considered to be significant for a confidence level of 95%.

RESULTS

Table II shows the different genotypes of the APOE gen as well as a comparison between patients and the control group. Table III presents the significance degree of allele $\epsilon 4$ in patients against the control group and the result of calculating the association degree (OR) of this allele with the group of patients vis-à-vis the control group. The risk depending of allele $\epsilon 4$ indicates that the probability of suffering AMD increases in a factor of 5.2 (IC 95%= 3.5-8.3; $p < 0.001$) for each allele $\epsilon 4$ the patient has. Allele $\epsilon 4$ is associated to AMD in a similar way in men (OR=5.25; IC 95%= 2.60-11.30) and in women (OR=5.55; IC 95%= 2.89-10.90). The association of allele $\epsilon 4$ with AMD is significant in all age groups: 65-70 years: OR=10.5; IC 95%= 4.89-22.90; 70-75 years: OR=7.15; IC 95%= 2.6-20.1; 75-80 years: OR=11.5; IC 95%= 4.89-26.90.

A stratified analysis of the sample by gender shows that allele $\epsilon 4$ is associated to a lower AMD diagnostic age in the group of women ($\epsilon 4+$ = 72.2 D.E. 5.1; $\epsilon 4-$ = 78,8 D.E. 5.7; $p < 0.001$).

DISCUSSION

Age-related macular degeneration is the first cause of irreversible blindness in Western countries.

Table II. APOE gen genotypes

	Control group	AMD group
Genotypes		
2/2	0	0
2/3	10 (15.3)	12 (12.6)
2/4	2 (3)	12 (12.6)
3/3	41 (63)	34 (35.7)
3/4	12 (18.4)	29 (30.5)
4/4	0	8 (8.4)

APOE: apolipoprotein E; AMD: age-related macular degeneration.

Table III. Allele $\epsilon 4$ and degree of association with AMD against controls

	% de APOE	OR	IC
Control	12%		
AMD	50%	5.6	3.4-8.8 ($p < 0.001$)

APOE: apolipoprotein E; OR: odds ratio; IC: Confidence interval; AMD: age-related macular degeneration.

The prevalence of late AMD is of 0.2% in the 55-64 age group, increasing up to 13% in the 85-year age group (1). It is estimated that the number of patients with AMD will double by the year 2020 (1). At present, therapeutic options for AMD are exclusively limited to the neovascular form with low success rates. From the viewpoint of public health there are preventive recommendations: no smoking, avoid prolonged exposure to sunlight, regularly eat greens and blue fish (1,3-4). However, it is difficult to establish the efficacy of these recommendations, which should be particularly emphasized for genetic risk subjects: family forms of AMD and some phenotype of APOE. AMD is a multifactorial pathology in which studies emphasise the importance of genetic factors (1,7-14). Recently, Hageman et al (7) in a study of F-factor gene (HF1/CFH) in a population of 900 patients with AMD and 400 controls, demonstrated the association between AMD and predisposition haplotypes (HF1 SNPs) and protection haplotypes. Klein et al found an association between AMD and variants in the F-factor gene, and accordingly homozygote individuals for the risk allele have an increased probability of 7.4 for suffering AMD (8).

In this study we aimed at studying the relationship of the apolipoprotein E gen genotype and AMD in a group of patients against a control group. The latter, being made up by spouses of AMD patients, minimized the effect of environmental and socio-economic variables which may influence the expression of APOE (16). In our sample (West Mediterranean) the relationship between allele $\epsilon 4$ and sporadic AMD is confirmed, and its presence increases five times the risk of suffering said disease. As regards gender, no differences have been appreciated in the degree of association between allele $\epsilon 4$ and the risk of AMD, and therefore the APOE genotype in our population would not be the basis for the greater prevalence of AMD in women. For this reason, other factors must be analysed such as longevity or hormonal alterations. Allele $\epsilon 4$ as a significant relationship with age in all groups, but the biggest relationship is in the 70-75 group. We have no explanation for the association found between allele $\epsilon 4$ and an earlier expression of AMD in women. Estrogens could be related to this difference, because it is known they interact with APOE at the level of the brain and are involved in the synthesis of the beta-amyloid peptid by microglya (17,18).

Clinical studies have provided contradictory results for the association between AMD and different APOE gen alleles (12-15). The result of this study is opposite to that of the majority of studies which demonstrated the protecting role of allele $\epsilon 4$ in AMD (7-14). Therefore, the reasons why this allele is a risk factor for cardiovascular diseases and Alzheimer (EA) (1,17,19) and not for AMD is an enigma, considering moreover that it has some histopathological similarities with EA (1). In summary, our study proves a non-protecting association of allele $\epsilon 4$ with AMD. We do not recommend the use of the APOE genotype as a AMD diagnostic test, but we consider it is important to establish intervention groups with prevention measures from the onset of the first symptoms.

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