Apolipoprotein E Genotype Is Associated with Macular Pigment Optical Density

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Purpose. Age-related macular degeneration (AMD) is the most common cause of blindness in older people in developed countries, and risk factors for this condition may be classified as genetic and environmental. Apolipoprotein E is putatively involved in the transport of the macular pigment (MP) carotenoids lutein (L) and zeaxanthin (Z) in serum and may also influence retinal capture of these compounds. This study was designed to investigate the relationship between macular pigment optical density (MPOD) and *ApoE* genotype.

Methods. This was a cross-sectional study of 302 healthy adult subjects. Dietary intake of L and Z was assessed by food frequency questionnaire, and MPOD was measured by customized heterochromatic flicker photometry. Serum L and Z were measured by HPLC. *ApoE* genotyping was performed by direct polymerase chain reaction amplification and DNA nucleotide sequencing from peripheral blood.

Results. Genotype data were available on 300 of the 302 (99.3%) subjects. The mean (± SD) age of the subjects in this study was 47.89 ± 11.05 (range, 21–66) years. Subjects were classed into one of three *ApoE* genotype groups, as follows: group 1, $\varepsilon2\varepsilon2$ or $\varepsilon2\varepsilon3$; group 2, $\varepsilon3\varepsilon3$; group 3, $\varepsilon2\varepsilon4$ or $\varepsilon3\varepsilon4$ or $\varepsilon4\varepsilon4$. All three groups were statistically comparable in terms of age, sex, body mass index, cigarette smoking, and dietary and serum levels of L and Z. There was a statistically significant association between *ApoE* genotype and MPOD. Subjects who had at least one $\varepsilon4$ allele had a higher MPOD across the macula than subjects without this allele (group 1 MPOD area, 0.70 ± 0.40; group 2 MPOD area, 0.67 ± 0.42; group 3 MPOD area, 0.85 ± 0.46; one-way ANOVA, $P = 0.014$.

Conclusions. These results suggest that *ApoE* genotype status is associated with MPOD. This association may explain, at least in part, the putative protective effect of the $\varepsilon4$ allele for AMD and is consistent with the view that apolipoprotein profile influences the transport and/or retinal capture of circulating L and/or Z. (*Invest Ophthalmol Vis Sci.* 2010;51:2636–2643) DOI:10.1167/iovs.09-4397

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Age-related macular degeneration (AMD) is the leading cause of blindness in people older than 50 years of age in the developed world and results in loss of central and color vision if not treated or if not amenable to treatment.[1–3] The prevalence of this condition is likely to increase dramatically in the future as a result of increasing life expectancy and the consequential increasing senescence of society.[4] The pathogenesis of AMD is incompletely understood, but it is believed to involve a complex interaction between multiple risk genes and environmental factors.[5,6]

Macular pigment (MP) is composed of the hydroxycarotenoids lutein (L), zeaxanthin (Z), and *meso*-zeaxanthin (*meso*-Z). L and Z are of dietary origin and are not synthesized de novo in humans, whereas *meso*-Z is not found in a conventional western diet but is understood to be formed primarily in the retina after conversion from L.[7,8] Interestingly, it has been shown that L is the dominant carotenoid in the diet,[9] whereas Z/*meso*-Z have been shown to be the dominant carotenoids at the central macula.[10,11] MP is found in highest concentration at the central macula, where it functions as a powerful antioxidant and acts as a filter of actinic short-wavelength blue light, thus limiting photo-oxidative damage to retinal cells.[12] These properties of MP are believed to be the mechanism whereby it may protect against the development and/or progression of AMD.

Although MP is entirely of dietary origin, it is also subject to heritability, as reported in 2005 by Liew et al.[13] in a classic twin study. In that study of 76 monozygotic and 74 dizygotic female twin pairs, they measured MP using two different techniques, heterochromatic flicker photometry (HFP) and fundus autofluorescence. They then applied genetic modeling techniques to determine the relative contributions of genetic and environmental factors to the variation in MP levels, estimating that heritability accounted for between 67% and 85% of a subject's MP level. Taken together with the recent finding of a relative lack of MP in association with a family history of AMD, it would appear reasonable to hypothesize that genetic predisposition to this disease may be attributable, at least in part, to a parallel and relative lack of this pigment.[6]

ApoE gene status is believed to be a determinant of AMD risk.[14–18] The *ApoE* gene has three separate alleles—Apo $\varepsilon2$, Apo $\varepsilon3$, and Apo $\varepsilon4$—which are differentiated on the basis of cysteine-arginine residue interchanges at positions 112 and 158 in the amino acid sequence.[19] As a result of this polymorphism, six common phenotypes exist: three homozygous phenotypes ($\varepsilon3\varepsilon3$, $\varepsilon2\varepsilon2$, $\varepsilon4\varepsilon4$) and three heterozygous phenotypes ($\varepsilon2\varepsilon3$, $\varepsilon2\varepsilon4$, $\varepsilon3\varepsilon4$). The $\varepsilon4$ allele has been found to be associated with a reduced risk for AMD, whereas the $\varepsilon2$ allele has been associated with an increased risk for this disease.[14–18,20,21] The *ApoE* gene codes for apolipoproteins, which are major transporters of lipids and cholesterol in the nervous system.[22]

It is known that L and Z are transported in serum on circulating lipoproteins and are relatively equally distributed between low-density lipoprotein (LDL) and high-density lipoprotein (HDL) molecules.[23–25] Because apolipoprotein E is

an integral part of chylomicrons and other lipoproteins, including very low-density lipoprotein (VLDL) and HDL,[19,26] we hypothesized that there may be a relationship between the quantity of MP in the retina and the apolipoprotein E profile, coded for by *ApoE* genotype. Moreover, the recent finding of a relative lack of MP in association with a clinically confirmed family history of AMD and the observation that MP optical density (MPOD), though entirely of dietary origin, is subject to heritability, prompted us to explore the relationship between this putatively protective pigment and *ApoE* genotype.[6,13]

Subjects and Methods

Subjects

Three hundred two subjects were recruited for this study, which was carried out in the Macular Pigment Research Group laboratory at the Waterford Institute of Technology (Waterford, Ireland). Subjects were recruited after local advertisement in various media. This study was approved by the Research Ethics Committee of the Waterford Institute of Technology, and each subject was required to sign an informed consent document before participation. All experimental procedures adhered to the tenets of the Declaration of Helsinki.

Inclusion criteria for participation in this study were age between 20 and 70 years, no clinical evidence of ocular pathology, no dietary supplementation with the MP carotenoids, and visual acuity of 20/40 or better. The following information was recorded for each subject: demographic details; family history of AMD (confirmed by letter from the diagnosing ophthalmologist); personal smoking history; and dietary intake of L and Z, assessed using a validated 170-item food frequency questionnaire (FFQ). Examination included visual acuity (Snellen and LogMAR); body mass index (BMI; calculated in kg/m^2); MPOD measurement by customized heterochromatic flicker photometry (cHFP; using the macular densitometer); nonmydriatic fundus photography, using a nonmydriatic auto fundus camera (AFC-210; Nidek, Aichi, Japan) to screen for ocular pathology; and 12-hour fasting blood samples taken to quantify serum concentrations of L and Z using high-performance liquid chromatography (HPLC) and for genotyping.

Food Frequency Questionnaire

Dietary intake of L and Z was assessed by a self-administered, semiquantitative FFQ developed by the Scottish Collaborative group (SCG; University of Aberdeen, Scotland, UK). This semiquantitative FFQ is the primary method used in epidemiologic studies to assess dietary intake of various nutrients and foods most commonly consumed in a Western diet.[27] This FFQ is a development of FFQs used in the Scottish Heart Health Study. It has been validated against weighed food records and biomarkers, and its validity, in terms of separately quantifying dietary intake of L and Z, has been confirmed in a study by O'Connell et al.[28–30]

The questionnaire is designed to assess a subject's dietary intake over the preceding 2 to 3 months. Although subjects were assessed at different time points throughout the year, it has been shown that month-to-month serum concentrations of L and Z and MPOD are relatively stable over a 24-month period.[31] The FFQ consists of 170 foods and drinks, grouped into 21 sections. A standard portion or measure for each type of food or drink is specified, and subjects are required to indicate how many portions they consumed per day and how often they consumed each type of food, ranging in frequency from "rarely or never" to "7 days per week." The questionnaire includes a color photograph depicting examples of standard food measures and an example of how to fill out the questionnaire. The questionnaire was completed by the subject in the presence of the primary investigator (EL) after detailed instructions for the required task. This questionnaire took between 20 and 30 minutes to complete.

Completed FFQs were inputted into a spreadsheet (Access; Microsoft, Redmond, CA) developed for analysis purposes by the SCG. Nutrient analysis was performed with a program (Visual Basic for Microsoft Access; Microsoft) using food composition data based on *McCance and Widdowson's The Composition of Foods*.[32] Dietary intake of L and Z was calculated based on food composition data from United Kingdom, European, and United States data sources according to standard principles or criteria for the matching of food items and standardized recipes or manufacturers' ingredient information where necessary.[33–36]

Measurement of Macular Pigment Optical Density

MPOD was measured psychophysically by cHFP, a technique that has been validated against the absorption spectrum of MP in vitro.[37] HFP is based on the fact that MP absorbs short-wavelength blue light, with peak absorption occurring at a wavelength of 458 nm. The subject is required to make isoluminance matches between two flickering lights, a green light (not absorbed by MP) and a blue light (maximally absorbed by MP). The log ratio of the amount of blue light absorbed centrally, where MP peaks, to that absorbed at a peripheral retinal locus (the reference point), where MPOD is assumed to be zero, gives a measure of the subject's MPOD.

In this study, we used the macular densitometer, a cHFP instrument slightly modified from a device described by Wooten et al.[38] Each subject was required to observe a flickering target, alternating in square-wave counterphase between a green light with a wavelength of 564 nm and a blue light with a wavelength of 460 nm and to make isoluminance matches between these flickering lights. The luminance of the green and blue lights is varied in a yoked manner, which avoids a change in the overall luminance of the test target. When an isoluminant (null flicker) match has been made between these flickering lights, flicker is no longer perceived; this is the desired end point of the test. Differently sized targets enable measurements of MPOD at 0.25°, 0.5°, 1°, and 1.75° retinal eccentricity, compared with a reference point at 7° retinal eccentricity (where MPOD is assumed to equal 0). Targets are presented on a blue background test field (wavelength, 468 nm) that saturates the S-cone pathway. A minimum of three null-flicker readings were recorded for each subject at each of the test loci (0.25°, 0.5°, 1°, 1.75°, and 7° retinal eccentricity). Measurement of MPOD at these points of retinal eccentricity enabled us to plot the spatial profile of MP across the macula. For each subject, we then calculated the area of MPOD under the spatial profile, using the trapezoid rule, as follows: MPOD area = ({[(MPOD at 0.25° + MPOD at 0.5°)/2] × 0.25°} + {[(MPOD at 0.5° + MPOD at 1°)/2] × 0.5°} + {[(MPOD at 1° + MPOD at 1.75°)/2] × 0.75°} + {[(MPOD at 1.75° + MPOD at 7°)/2] × 5.25°}), assuming an MPOD of zero at 7° retinal eccentricity and also assuming a linear fit between each successive point of measurement of MPOD but a nonlinear MP spatial profile overall, as illustrated for subject 1 in Figure 1. This MPOD area gives a better measurement of the quantity

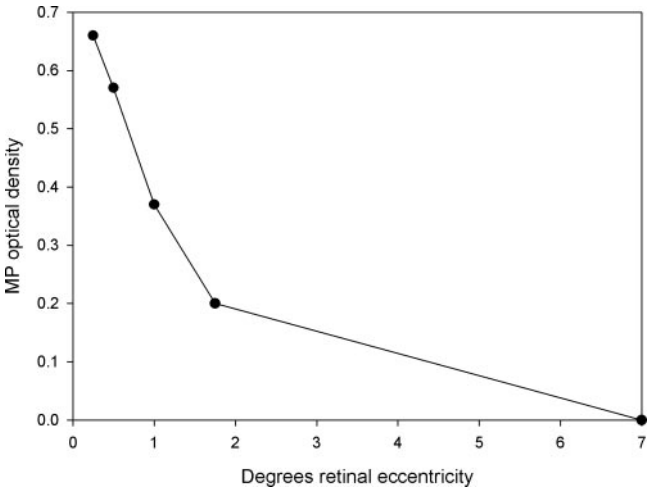

Figure 1. MPOD spatial profile for subject 1.

of MP across the macula than an individual measurement at a single point of retinal eccentricity. MPOD measurement was performed under conditions of dimmed light (ambient illuminance 4 lux, as measured with a lux meter [ILM 350; Iso-Tech, Champaign, IL]) at a viewing distance of 18.5 inches (47 cm).

The major advantage of cHFP over standard HFP instruments is that the flicker frequency of each test target is customized for each subject, minimizing the variance between consecutive measurements and, thus, increasing the accuracy and ease of use of the test. A more detailed description of this instrument has been published by Wooten et al.[38] Further information on the technique and advantages of cHFP have been published by Nolan et al.[39] and Loane et al.[40]

Blood Sample Collection

A 12-hour fasting blood sample was collected from each subject in a 4-mL tube (Z Serum Sep Clot Activator Vacuette; Greiner Bio-One GmbH, Kremsmünster, Austria) at the beginning of the study visit. This whole blood sample was immediately refrigerated at 2°C to 8°C, before centrifugation at approximately 1800*g* for 10 minutes. Centrifugation was performed within 4 hours of phlebotomy. After centrifugation, the supernatant serum sample was divided as aliquot portions into 1.5-mL amber light-sensitive microcentrifuge tubes (Brand GmbH, Wertheim, Germany) and stored at minus 70°C before analysis.

Serum L and Z Analyses

Serum L and Z were quantified using reversed-phase HPLC. We used an Agilent system (1200 series LC; Agilent Technologies Ireland Ltd., Dublin, Ireland), with photodiode array detection at 295 nm, 325 nm, and 450 nm. A 5-μm analytical/preparative 4.6 $\times$ 250 mm 201TP specialty reversed-phase column (Vydac, Hesperia, CA) was used with an in-line guard column. The mobile phase consisted of 97% methanol and 3% tetrahydrofuran and was degassed using an in-line degasser. The flow rate was 1 mL/min, and the total run time was 15 minutes. All carotenoid peaks were integrated and quantified using corresponding software (Chem Station; Agilent).

A 400-μL aliquot of serum was pipetted into a 1.5-mL clear microcentrifuge tube. Ethanol (300 μL) containing 25 μg/mL butylated hydroxytoluene and 200 μL internal standard containing 25 μg/mL α-tocopherol acetate was added to each tube. Heptane (500 μL) was then added, and samples were vortexed vigorously for 1 minute, followed by centrifugation at approximately 225*g* for 5 minutes. The resultant heptane layer was retained and transferred to a second, labeled 1.5-mL light-sensitive microcentrifuge tube, and another heptane extraction was performed. The combined heptane layers were immediately evaporated to dryness under a stream of nitrogen using a sample concentrator (Techne Sample Concentrator; Davison & Hardy Ltd., Belfast, Northern Ireland, UK). These dried samples were reconstituted in methanol (200 μL), and a 150-μL sample was injected for HPLC analysis.

DSM Nutritional Products (Basel, Switzerland) provided the L and Z standards that were used to generate standard curves for quantification of these carotenoids. This assay was validated against the National Institute of Standards and Technology standard before analysis.

ApoE Sequencing

DNA was extracted from peripheral blood leukocytes or frozen buffy coat samples using standard protocols. The *ApoE* subtypes are determined by the combination of alleles at the two nonsynonymous SNPs, rs429358 (C130R) and rs7412 (R176C), located within exon 4. Exon 4 was PCR amplified using sense (CCTCTGCCCCGTTCCTTCTC) and antisense (GGCCAGCAGATGCGTGAAAC) oligonucleotides under the following PCR conditions: 95°C for 60 seconds, followed by 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 60 seconds, followed by 72°C for 10 minutes. A sequencing kit (Big Dye Terminator v3.1 Cycle ABI Prism; Applied Biosystems, Foster City, CA) was used in conjunction with the *ApoE* antisense sequencing primer (TCCTGTAGCGGCTGGCCG) according to the manufacturer's instructions for processing on a genetic analyser (ABI 3100; Applied Biosystems).

Statistical Analysis

A commercially available statistical software package (SPSS version 17; SPSS, Chicago, IL) was used for statistical analysis of results. A graphics software package (SigmaPlot version 8.0; Systat, San Jose, CA) was used for graphical presentation of results. One-way ANOVA was used to investigate differences in variables between the various genotype groups. Further associations were investigated using a general linear model approach, with Pearson chi-square testing and independent-samples *t* testing, as appropriate. The level of statistical significance was set at the standard $P < 0.05$.

Results

Genotype data were available on 300 of the 302 (99.3%) subjects included in this study. We divided the study sample into three *ApoE* genotype groups (group 1, $\varepsilon2\varepsilon2$ or $\varepsilon2\varepsilon3$; group 2, $\varepsilon3\varepsilon3$; group 3, $\varepsilon2\varepsilon4$ or $\varepsilon3\varepsilon4$ or $\varepsilon4\varepsilon4$) for analysis purposes, as detailed in Table 1. This was done to increase the statistical power of our analysis because there were very few subjects with certain genotypes given the relatively low minor allele frequency (MAF) of rs429358 (MAF, 0.11) and rs7412 (MAF, 0.09) in Caucasian populations (one subject had the $\varepsilon2\varepsilon2$ genotype, two subjects had the $\varepsilon2\varepsilon4$ genotype, and six subjects had the $\varepsilon4\varepsilon4$ genotype). Interestingly, the subject with the $\varepsilon2\varepsilon2$ genotype had a clinically confirmed family history of AMD, and 5 of 6 (83.3%) subjects with the $\varepsilon4\varepsilon4$ genotype had a negative family history of AMD.

Anthropometric and lifestyle data of all subjects, divided according to *ApoE* genotype group, are detailed in Table 2. The mean ($\pm$ SD) age of all subjects who underwent genotyping in this study was 47.89 $\pm$ 11.05 (range, 21–66) years. Seventy percent of the subjects included in this study were women. There was no statistically significant difference between each *ApoE* genotype group in terms of any of the following variables: age, BMI, cigarette smoking, dietary L intake, dietary Z intake, serum L concentration, serum Z concentration (one-way ANOVA, $P > 0.05$). There was also no statistically significant difference between the *ApoE* genotype groups in terms of the male/female ratio of subjects or between the ratio of subjects with a positive/negative family history of AMD in each of the *ApoE* genotype groups (Pearson chi-square $P > 0.05$, for both comparisons; Table 1).

MPOD at 0.5° retinal eccentricity of all genotyped subjects was 0.38 $\pm$ 0.17 (range, 0–1.02 optical density units [ODU]). There was a statistically significant association between *ApoE* genotype and MPOD at all degrees of retinal eccentricity (one-way ANOVA, $P \leq 0.016$), except at 1.75°, where it approached statistical significance (one-way ANOVA, $P = 0.062$); subjects who had at least one $\varepsilon4$ allele had higher MPOD than subjects without this allele. There was also a statistically significant association between MPOD area and *ApoE* genotype (one-way ANOVA, $P = 0.014$), as illustrated in Figure 2.

Table 1. *ApoE* Genotype Distribution for All Subjects

ApoE Genotype	Entire Group (n = 300)	Negative Family History (n = 179)	Positive Family History (n = 121)
Group 1 ($\varepsilon2\varepsilon2$, $\varepsilon2\varepsilon3$)	40	25	15
Group 2 ($\varepsilon3\varepsilon3$)	190	113	77
Group 3 ($\varepsilon2\varepsilon4$, $\varepsilon3\varepsilon4$, $\varepsilon4\varepsilon4$)	70	41	29

TABLE 2. Anthropometric and Lifestyle Data for All Subjects According to *ApoE* Genotype Group

Characteristic	Group 1 (n = 40)	Group 2 (n = 190)	Group 3 (n = 70)
Age, y	49.20 ± 10.80	48.37 ± 11.50	45.83 ± 9.76
Sex, n %			
Male	12 (30)	54 (28.4)	24 (34.3)
Female	28 (70)	136 (71.6)	46 (65.7)
BMI*	26.29 ± 3.92	26.79 ± 4.71	26.64 ± 4.52
MPOD† 0.25°	0.50 ± 0.24	0.45 ± 0.20	0.56 ± 0.19
MPOD 0.5°	0.38 ± 0.17	0.36 ± 0.17	0.43 ± 0.18
MPOD 1°	0.24 ± 0.12	0.22 ± 0.12	0.29 ± 0.14
MPOD 1.75°	0.12 ± 0.09	0.12 ± 0.09	0.15 ± 0.11
MPOD area	0.70 ± 0.40	0.67 ± 0.42	0.85 ± 0.46
Smoking, pack-years‡	5.33 ± 13.76	5.69 ± 10.35	5.82 ± 12.40
Dietary L, mg	1.376 ± 1.695	1.421 ± 1.113	1.234 ± 0.857
Dietary Z, mg	0.189 ± 0.150	0.189 ± 0.107	0.181 ± 0.091
Serum L, μg/mL	0.085 ± 0.047	0.089 ± 0.047	0.092 ± 0.049
Serum Z, μg/mL	0.016 ± 0.014	0.017 ± 0.017	0.017 ± 0.010
Positive family history of AMD, %	37.5	40.5	41.4

Group 1, ε2ε2 *or* ε2ε3; Group 2, ε3ε3; Group 3, ε2ε4 *or* ε3ε4 *or* ε4ε4.

* Body mass index in kilograms divided by height in meters squared (kg/m^2).

† Differences between groups are significant at the $P = 0.05$ level for all MPOD values except at 1.75° eccentricity. The observed differences are between group 3 and the other two groups. There is no statistically significant difference between group 1 and group 2.

‡ Pack-year calculation = (no. cigarettes smoked per day × no. years smoking)/20.

When we analyzed subjects with the ε4ε4 genotype as a separate group, we again found a statistically significant association between *ApoE* genotype and MPOD at all degrees of retinal eccentricity (one-way ANOVA, $P \leq 0.028$), except at 1.75° (one-way ANOVA, $P = 0.135$); subjects who had the ε4ε4 genotype had significantly higher MPOD than other subjects, as detailed in Table 3. The statistically significant association between MPOD area and *ApoE* genotype (one-way ANOVA, $P = 0.036$) is illustrated in Figure 3.

DISCUSSION

This study investigated the relationship between MPOD and *ApoE* genotype in 302 healthy subjects between 21 and 66 years of age. The mean MPOD of all genotyped subjects at 0.5° retinal eccentricity was 0.38 ± 0.17 ODU, which is comparable to that in previous studies that used HFP to measure MPOD at this eccentricity.[6,41–45] Genotype data were available on 99.3% of our sample.

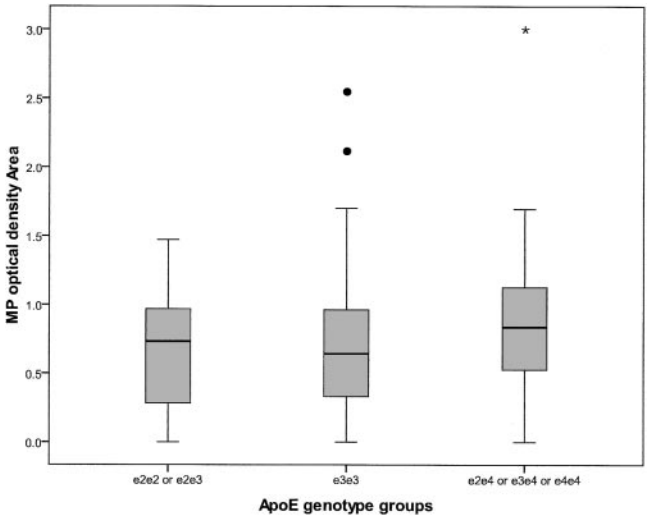

FIGURE 2. Box plots showing MPOD area versus *ApoE* genotype for all subjects.

It is widely accepted that the effect exerted by the ε3 allele is neutral for a number of diseases and that the effects exerted by either the ε2 or the ε4 allele are either risky or protective, depending on the disease in question.[46–48] In the case of *ApoE* and AMD, most studies, but particularly the more robust meta-analyses, have suggested that the ε4 allele acts in a dominant manner and that it has a protective effect regardless of the presence of one or two alleles.[48] Therefore, although it would have been preferable to compare samples of equal sizes, this was not possible because of the low in vivo haplotype frequencies of the ε2 and ε4 alleles in the population under investigation. As a result, we elected to divide the study sample into three genotype groups for purposes of analysis.

We found that there was a statistically significant association between MPOD and *ApoE* genotype. Subjects who carried the ε4 allele had a higher MPOD than did noncarriers; the mean difference in the MPOD area between the ε4 allele group (group 3) and the other two groups was 0.18 ODU. This difference could not be attributed to differences in dietary intake or differences in serum concentrations of the macular carotenoids among the three groups. Importantly, the three analysis groups were also comparable to one another with respect to age, sex, BMI, and cigarette smoking, factors that may influence MPOD.[6] We did not observe a relative lack of MPOD in association with the ε2 allele, which is putatively, but unconvincingly, linked to an increased risk for AMD compared with the ε3 allele. However, in the three meta-analyses investigating the association between *ApoE* and AMD, an increased odds ratio between 1.11 and 1.33 was observed in association with the ε2 allele; this finding was nonstatistically significant in one of these meta-analyses and barely significant in the other two.[48–50]

MP carotenoids are derived entirely from diet, but macular levels of these carotenoids are subject to influence by genetic background, as shown in the classic twin study by Liew et al.[13] There is a large body of evidence to suggest that MP may protect against the development and/or progression of AMD.[51] This putative protective effect is based on the known properties of MP as a prereceptoral filter of actinic short-wavelength blue light and its antioxidant capacity, including the ability to quench singlet oxygen[52] and to inhibit the peroxidation of membranous phospholipids.[53] It has been estimated that MP

Table 3. MPOD Values at Each Retinal Eccentricity According to *ApoE* Genotype, with the ε4ε4 Genotype Analyzed Separately

Characteristic	Group 1 ε2ε2 *or* ε2ε3 (*n* = 39)	Group 2 ε3ε3 (*n* = 187)	Group 3 ε2ε4 *or* ε3ε4 (*n* = 63)	Group 4 ε4ε4 (*n* = 6)
MPOD 0.25°	0.50 ± 0.24	0.45 ± 0.20	0.55 ± 0.19	0.62 ± 0.26
MPOD 0.5°	0.38 ± 0.17	0.36 ± 0.17	0.42 ± 0.18	0.49 ± 0.22
MPOD 1°	0.24 ± 0.12	0.22 ± 0.12	0.29 ± 0.14	0.26 ± 0.16
MPOD 1.75°	0.12 ± 0.09	0.12 ± 0.09	0.15 ± 0.11	0.15 ± 0.06
MPOD Area	0.70 ± 0.40	0.67 ± 0.42	0.85 ± 0.47	0.87 ± 0.37

Values are expressed as mean ± SD. Differences between groups are significant at the $P = 0.05$ level for all MPOD values except at 1.75° eccentricity. The observed differences are between groups 3 and 4 and the other two groups. There is no statistically significant difference between group 1 and group 2 or between group 3 and group 4.

absorbs approximately 40% of damaging short-wavelength irradiation before its incidence on the photoreceptors and the retinal pigment epithelium (RPE).[54] This is deemed to be particularly important because it has been shown by Ham et al.[55] that exposure to short-wavelength blue light can result in photochemical retinal injury in primates. In their study, they exposed rhesus monkey retinas to blue light for 1000 seconds, which resulted in atrophic AMD-like lesions and damage to the photoreceptor outer segments, cellular proliferation, and hypopigmentation of the RPE. They found that the threshold for such retinal damage was lowest for blue light than for other wavelengths of visible light.[55] In addition, it has been shown that the administration of antioxidants can prevent light-induced retinal damage in rat retinas.[56] In other words, there is a substantial body of evidence that cumulative exposure to short wavelength blue light is involved in the pathogenesis of AMD and that this mechanism of retinal injury can be prevented by the administration of antioxidants.

Beyond the contribution of MP to limiting blue light–induced photo-oxidative damage and the consequential generation of reactive oxygen intermediates (ROIs), its constituent carotenoids are also known to quench existing ROIs in the retina as reported by Khachik et al.,[57] who demonstrated the presence of direct oxidation products of L and Z in the retina. Indeed, oxidatively damaged photoreceptors cannot be completely digested by the apposing RPE, thus contributing to the accumulation of lipofuscin in this apposing cell layer.[58] Importantly, lipofuscin is a chromophore, rendering the blue light–filtering properties of MP all the more important.[59,60] Indeed, it has been hypothesized that the relative lack of lipofuscin in foveal RPE cells, when compared with surrounding RPE cells, is the result of protection conferred by MP against oxidation of the overlying photoreceptors and a consequential reduction in lipofuscin production at this location.[61,62]

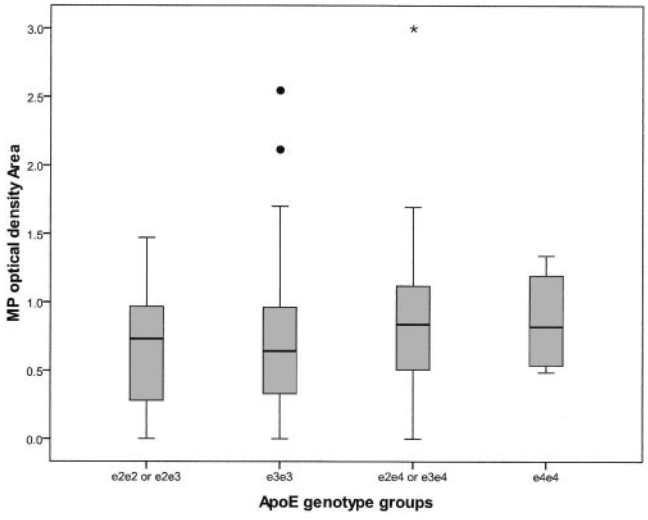

Figure 3. Box plots showing MPOD area versus *ApoE* genotype for all subjects.

The absorption of the MP carotenoids, L and Z, from the gastrointestinal tract involves incorporation into micelles,[23] most likely followed by a facilitated, protein-mediated transport mechanism across human enterocytes.[63] Once absorbed into the bloodstream, L and Z are associated with plasma lipoproteins, being relatively equally distributed between LDL and HDL,[23–25] with a progressive decrease in the content of L and Z from light to dense LDL. However, some studies have reported that HDL is the preferential carrier of the MP carotenoids in plasma.[64–66] The uptake of these carotenoids into the retina is less well understood. Bernstein et al.[67] and Bhosale et al.[68] have identified specific xanthophyll-binding proteins, of which the Pi isoform of glutathione S-transferase has a high affinity for Z and *meso*-Z, but not for L. In a recent publication, Bhosale et al.[68] have also identified a human retinal L-binding protein purified from peripheral human retina.[69] Connor et al.[65] have shown in a study of Wisconsin hypo-alpha mutant chicks, which have a genetic mutation resulting in very low circulating HDL levels, that retinal L accumulation was only 6% that of control chicks, suggesting an important role for HDL in the transport of L in serum, its capture by the retina, or both.

The *ApoE* gene codes for apolipoproteins, which are the protein constituents of lipoproteins, particularly plasma chylomicrons, VLDL, and a subclass of HDL. Apolipoprotein E is essential for cholesterol transport and metabolism and for receptor-mediated uptake of specific lipoproteins.[70] It is an important regulator of cholesterol metabolism because of its affinity for apolipoprotein E-specific receptors in the liver and its affinity for LDL receptors in the liver and other peripheral tissues requiring cholesterol.[70] Apolipoprotein E is polymorphic and three common isoforms—E2, E3, and E4—which are coded for by three separate alleles, Apo ε2, Apo ε3, and Apo ε4. These alleles are differentiated on the basis of cysteine-arginine residue interchanges at positions 112 and 158 in the amino acid sequence.[19] As a result of this polymorphism, six common phenotypes exist—three homozygous phenotypes (ε3ε3, ε2ε2, ε4ε4) and three heterozygous phenotypes (ε2ε3, ε2ε4, ε3ε4). *ApoE* polymorphisms result in differences in the metabolism of apolipoprotein E-containing lipoprotein particles.[71]

In the retina, apolipoprotein E is synthesized in Müller cells and in the RPE, and apolipoprotein E has been identified in drusen.[72–74] Klaver et al.[15] suggested that a high degree of apolipoprotein E biosynthesis is required to support the high rate of photoreceptor renewal in the macular region. Indeed, clinical manifestations of retinal degeneration are exhibited in *ApoE*-deficient mice that carry an *ApoE* gene inactivated by

gene targeting.[75] However, the association of the *ApoE* gene with AMD in humans has been inconsistent, with some studies showing a protective effect for the ε4 allele and an increased risk in carriers of the ε2 allele,[14–17,21,49] whereas others have shown no such association with the disease.[76–82] In the three meta-analyses that have been reported on the association between *ApoE* profile and AMD, a decreased odds ratio associated with the ε4 allele, ranging from 0.6 to 0.67, was observed, and this association was highly significant in all three meta-analyses.[48–50] Thus, the evidence to date favors a protective role for the Apo ε4 allele because several of the studies that did not show an overall association between the *ApoE* gene and AMD have reported a nonsignificant trend toward a protective effect for this allele.

Selective binding of certain receptors to HDL particles enriched with apolipoprotein E has been demonstrated within the CNS.[83] These receptors include the LDL receptor and the LDL receptor-related protein. It has also been shown that there is a lack of binding of such receptors to HDL particles deficient in apolipoprotein E. This selectivity of the uptake mechanism may be dependent on the specific apolipoprotein E that forms part of the transporting lipoprotein molecule. This, in turn, could affect the ability of retinal receptors to bind with, and incorporate, such molecules, thus affecting the uptake of any other substances transported by these lipoproteins. Because Connor et al.[65] have demonstrated that retinal uptake of L is facilitated by an HDL-mediated transport system, it is reasonable to suggest that apolipoprotein E polymorphism may impact the method of capture of L by the retina. Certainly, our observation that the Apo ε4 allele is associated with a relative surplus of MP, but in a way that is unrelated to dietary intake or serum levels of its constituents, supports this view and represents a possible explanation for the putative protective role that the Apo ε4 allele plays in the pathogenesis of AMD.

In conclusion, we have shown that there is a statistically significant and positive association between MPOD and presence of at least one Apo ε4 allele in a cohort of 300 healthy subjects. This observation may relate to the role that apolipoprotein E4, coded for by the Apo ε4 allele, plays in the lipoprotein-mediated transport of L and Z in serum and the subsequent uptake of these carotenoids by the retina.

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