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Assessment of macular pigment optical density (MPOD) in patients with unilateral wet age-related macular degeneration (AMD)

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ABSTRACT.

Purpose: **To compare the macular pigment optical density (MPOD) of patients with unilateral wet age-related macular degeneration (AMD) with the MPOD of bilateral dry AMD patients and healthy elderly individuals.**

Methods: **The MPOD of 34 patients with unilateral wet AMD was measured in their fellow eye that had the dry form of the disease (study group). The MPOD of the study group was compared with the MPOD of 33 patients with bilateral dry AMD (patients' control group) and 35 elderly subjects without any signs of retinal disease (control group). None of the subjects was under carotenoid supplementation. The MPOD was measured with Heterochromatic Flicker Photometry [QuantifEYE™ – MPS 9000 (ZeaVision©)]. The statistical package SPSS v 17.0 was used for the analysis.**

Results: **The overall mean MPOD was 0.52 (SD 0.15). Patients with unilateral wet AMD have significantly higher levels of MPOD in their fellow eye compared with patients with bilateral dry AMD (0.58 versus 0.48, p = 0.026). Mean MPOD of patients with bilateral dry AMD does not differ significantly from that of healthy elderly subjects (0.48 versus 0.50, p = 0.865). In this population sample, no correlation with age was observed, while women have slightly but significantly higher levels of MPOD (0.55 versus 0.49, p = 0.029).**

Conclusion: **In the present study, the mean MPOD at the fellow eye of patients with unilateral wet AMD was found to be significantly higher than that of patients with bilateral dry AMD, while no other significant difference emerged between groups. Further investigation is demanded to clarify the role of macular pigment in AMD progression.**

Key words: age-related macular degeneration – heterochromatic flicker photometry – lutein – macular pigment optical density – wet AMD – zeaxanthin

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Introduction

Lutein and zeaxanthin are the main constituents of the macular pigment (MP), a yellowish substance that extends throughout the macula (Bone et al. 1988). Both substances have strong antioxidant properties (Snodderly 1995, Stahl & Sies 2003), and they also act as a 'blue filter' (Snodderly et al. 1984), protecting the retina from oxidative stress, both metabolic (Stahl et al. 1997) and photochemical (Snodderly et al. 1984; Sparrow & Cai 2001; Bone et al. 2003). These antioxidant properties implicated the MP in the research for the pathogenesis of age-related macular degeneration (AMD), where oxidative stress is suggested to play a major role (Beatty et al. 2000a,b).

There are several techniques for measuring MP levels at the macula, physical and psychophysical. Heterochromatic flicker photometry (Wooten et al. 1999; Bone & Landrum 2004; Snodderly et al. 2004), fundus autofluorescence (Delori et al. 2001 & Delori 2004) and fundus reflectance (Berendschot & van Norren 2004, 2005) are some of them, using a variety of methodologies. In our study, an HFP method was used that will be discussed in more details further after.

The first large study (N = 4757) that implied the potential benefit of the retinal carotenoids on the progression of AMD was the AREDS I in 2001 (Age-Related Eye Disease Study Research Group 2001a,b,c), while 7 years later, the Blue Mountains Eye Study confirmed these findings (Tan et al. 2008). Moreover, the AREDS I estimated the risk of developing advanced AMD in the fellow eye of patients with advanced AMD in one eye at 43% over 5 years, setting these patients in a high-risk group demanding close follow-up.

The effectiveness of antioxidant supplementation, including carotenoids, to retard the progression of AMD to advanced stages (Age-Related Eye Disease Study Research Group 2001a,b,c; Tan et al. 2008) could mean that the retina of these patients has lower antioxidant protection and possibly lower MP levels. Furthermore, one would expect that MP levels will further decline with the progression of the disease, as already suggested (Nolan et al. 2007). Based on this, we hypothesized that macular pigment optical density (MPOD) in patients with dry AMD in one eye and wet AMD in the fellow eye would be considerably low.

The purpose of this study was to compare MPOD of patients with wet AMD in one eye and dry AMD in the fellow eye with that of patients with bilateral dry AMD and healthy elderly subjects.

Material and Methods

The study adhered to the tenets of the Declaration of Helsinki.

Patients

Volunteers from the Ophthalmological Clinic of the University Hospital of Heraklion, Crete (OCUHHC), were recruited and formed the three groups of the study. The study group (Group 1) consisted of 34 patients with the exudative form of AMD in one eye and any stage of dry AMD in their fellow eye (AREDS I classification) (Age-Related Eye Disease Study Research Group 2001a,b,c). The eye with dry AMD was characterized as study eye. The second group (Group 2) included 33 patients with bilateral dry AMD both early and intermediate (stages 2 & 3 according to the AREDS I). In cases that both eyes were eligible for enrolment, the left eye was preferred. The third group (Group 3) comprised 35 subjects with no AMD in either eye. Again, if both eyes were eligible for enrolment, the left eye was preferred. Groups 2 and 3 served as control groups. Patients of Groups 1 and 2 were followed-up regularly at the Fundus Department of the OCUHHC, while Group 3 consisted of healthy elderly subjects programmed for cataract extraction and were all measured preoperatively.

The diagnosis of AMD was set and confirmed subjectively (clinical examination) and objectively [(optical coherent tomography (OCT), colour fundus photography (CF) and fluorescein angiography (FA)]. All 102 subjects underwent a complete ophthalmic evaluation including best corrected visual acuity (BCVA), using ETDRS charts, slit-lamp examination, tonometry and fundoscopy; all subjects have also had OCT (Stratus OCT™ v 4.0.5; Carl Zeiss Meditec Inc, Dublin, CA, USA) and CF. Additionally, all the patients of the study group (Group 1) have had FA (Retinal Camera TRC-50DX; Topcon Inc, Tokyo, Japan) for the confirmation of advanced AMD in the one eye and the lack of it in the fellow study eye. Dry AMD was categorized by fundoscopy and CF, according to the AREDS I classification (Age-Related Eye Disease Study Research Group 2001a,b,c).

The enrolment of subjects was made according to the following inclusion criteria. All recruited individuals should have BCVA equal or better than 20/80 (0.6 logMAR) in the study eye and should be able to complete a successful MPOD measurement as it is described below. Group 1 included patients with wet AMD in one eye and dry AMD in the fellow eye (early or intermediate, according to the AREDS I categorization). The MPOD was measured at the eye with dry AMD (study eye). Group 2 included patients with bilateral dry AMD both early and intermediate (stages 2 & 3 according to the AREDS). The MPOD was assessed in both eyes if they fulfilled the first criterion. Group 3 included elderly phakic subjects without AMD or other retinal pathology; again, the MPOD was measured in both eyes if possible. When both eyes could be enrolled, the left eye was preferred.

The exclusion criteria involved the intake of any supplement that contains the retinal carotenoids lutein and/or zeaxanthin and/or meso-zeaxanthin; the presence of advanced AMD (CNV or geographic atrophy) in the study eye of Group 1 and in any eye in Groups 1 and 3; the existence of any other form of retinopathy except for AMD.

MP evaluation

The macular pigment was quantified in terms of MPOD. The MPOD was measured psychophysically with the MPS 9000 (Macular Pigment Screener; Tinsley Ophthalmic Instruments, Redhill, Surrey, UK) that uses a modified HFP technique for its estimation. A detailed description of the device can be found in previous publications by Van der Veen et al. (2009) and Makridaki et al. (2009).

A typical measurement session includes two sets of measurements. Initially, observers are asked to fixate a central 1° target. The target is composed of two alternating LEDs; 'blue' (465 nm) and 'green' (530 nm) with luminance up to 200 cd/m^2. They are superimposed on a white light pedestal (colour temperature 5500 K). The blue LED is absorbed by the MP, while the green LED is absorbed negligibly. On each measurement, the two LEDs start to flicker at a rate of 60 Hz that gradually reduces at a rate of 6 Hz/second. The flickering rate of 60 Hz is above the critical flicker fusion rate; as a result, initially, the target appears static. Observers are asked to press the response button as soon as a flicker is detected. Measurements are obtained for a series of green–blue luminance ratios.

The same procedure is repeated for peripheral viewing of the target. Again, observers are asked to fixate a 2° red spot located at 8° horizontal eccentricity from the central target. Observers are advised to press the response button when they detect the flicker of the central target with their peripheral vision. They are advised to keep blinking during the procedure and not to stare intensively at the red target. After the end of the second part of the measuring session, MPOD calculation is based on the detection

of iso-luminant point while observed centrally, where MP is considered to be of maximum density and with peripheral vision where MP is expected to be absent. Each complete measuring session for the estimation of MPOD takes approximately 9–10 min per eye.

The MPOD measurement was performed in all patients. Measurements were made in a dark room with the fellow eye covered with an eye patch. The measurement was repeated three times, and the mean value of MPOD was finally recorded for the analysis.

Statistical analysis

Initially, univariate comparisons between Groups 1, 2 and 3 were made using the Chi-squared test of independence for the categorical variable sex, while one-way ANOVA was used to compare average ages and MPOD measurements between the groups of participants. *Post hoc* pairwise comparisons were undertaken using Scheffe contrasts. Subsequently, multiple linear regression (generalized linear modelling with an identity link) was used to assess the effect of AMD status on average MPOD levels, adjusting for the potential confounders' age and sex. The possibility of second-order interactions was considered. Information on smoking status and iris colour was available for 82 of 102 subjects. A further model was fitted which incorporated both smoking status and iris colour. The distribution of the residuals was used to assess model fit. The significance level chosen was 5%. The statistical package SPSS ver17.0 was used throughout.

Results

Fifty-two of the 102 participants were women (51%). The mean age of subjects was 72 years (SD 6.5, minimum 55 years, maximum 88 years). Average age, sex, iris colour and smoking status did not differ to a statistically significant extent between the three groups. Demographic characteristics of the participants by AMD status are presented in Table 1.

The overall mean MPOD level was 0.52 (SD 0.15, minimum 0.22, maximum 0.89). The mean MPOD of Group 1 (study group) was 0.58 (SD 0.14, minimum 0.29, maximum 0.82), Group 2 (bilateral dry AMD patients) 0.48 (SD 0.14, minimum 0.22, maximum 0.84) and Group 3 (healthy Controls) 0.50 (SD 0.14, minimum 0.24, maximum 0.89).

Mean MPOD was found to differ statistically between groups ($p = 0.016$). *Post hoc* tests revealed average MPOD to be *higher* in Group 1 (study group) compared with Group 2 (bilateral dry AMD) (difference in mean MPOD 0.094, SE 0.034, $p = 0.026$). The difference between Group 1 and Group 3 was not found to be statistically significant (difference in mean MPOD 0.076, SE 0.034, $p = 0.085$), neither between Group 2 and Group 3 (difference in mean MPOD 0.018, SE 0.034, $p = 0.865$).

Even after accounting for possible age and sex effects, average MPOD levels were found to differ to a statistically significant extent between the three groups ($p = 0.036$), as can be seen in Table 2. Figure 1 depicts the difference between means of MPOD among groups.

The second-order interactions between MPOD, sex and age were not found to be statistically significant and so were not considered further. In addition, women were found to have somewhat higher MPOD levels, on average, than men (0.059 units higher, 95% CI 0.005–0.112, $p = 0.031$). Figure 2 depicts the difference between men and women.

Iris colour and smoking status were not found to have a statistically significant effect, when AMD status, age and sex were included in the model.

Discussion

In the present study, we were interested specifically in patients with unilateral advanced (wet) AMD. These patients are practically monocular, as they have already very low vision in the one eye (with the advanced disease), and at the same time, they are at high risk to develop wet AMD in their fellow eye. The possibility of having MP values that could warn us about potential conversion of the

Table 1. Characteristics of study participants ($n = 102$) by AMD status.

	Group 1 (wet/dry AMD) ($n = 34$)	Group 2 (bilateral dry AMD) ($n = 33$)	Group 3 (no AMD) ($n = 35$)	p-value
Sex				
Males	13 (38%)	16 (49%)	21 (60%)	0.194*
Females	21 (62%)	17 (51%)	14 (40%)	
Iris colour				
Light	7 (27%)	8 (24%)	3 (14%)	0.519*
Dark	19 (73%)	26 (77%)	19 (86%)	
Smoking status‡				
Current or ex-smoker	11 (42%)	12 (35%)	12 (54%)	0.360*
Never smoker	15 (58%)	22 (65%)	10 (46%)	
Mean age in years (SD)	74.4 (6.4)	71.6 (6.6)	71.5 (6.2)	0.111†
Mean MPOD (SD)	0.58 (0.14)	0.48 (0.15)	0.50 (0.14)	0.016†

* p-Values come from Pearson's X^2 statistic while for.
† p-Values come from one-way ANOVA.
‡ The information was only available for 82 subjects in total (20% missing).
AMD, age-related macular degeneration; MPOD, macular pigment optical density.

Table 2. Regression coefficients for the linear regression of MPOD on AMD status, age and sex.

	Regression coefficient (95% CI)	p-value
Constant	0.356 (0.053 to 0.660)	0.021
Group*		0.036
3 (no AMD)	0.025 (−0.039 to 0.090)	0.442
1 (Study group)	0.085 (0.019 to 0.151)	0.012
Age (years)	0.001 (−0.003 to 0.006)	0.529
Female subject†	0.059 (0.005 to 0.112)	0.031

* Reference category = subjects with bilateral dry AMD (Group 2).
† Reference category = male subjects.
AMD, age-related macular degeneration; MPOD, macular pigment optical density.

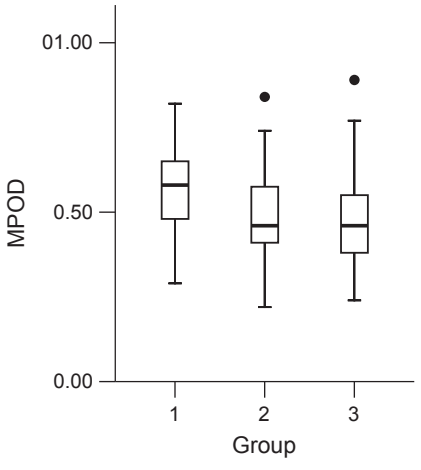

Fig. 1. Boxplots depicting mean macular pigment optical density (MPOD) of the three groups of the study. (1): MPOD of the fellow eye of patients with unilateral wet age-related macular degeneration (AMD) (Study Group), (2): MPOD of patients with bilateral dry AMD and (3): MPOD of healthy elderly controls. Means: (1): 0.58 (SD 0.14, minimum 0.29, maximum 0.82), (2):0.48 (SD 0.14, minimum 0.22, maximum 0.84), (3): 0.50 (SD 0.14, minimum 0.24, maximum 0.89). Mean MPOD differs significantly between groups ($p = 0.016$). Study group has significantly higher MPOD level than Group 2 ($p = 0.026$. SE 0.034), but not from Group 3 ($p = 0.085$, SE 0.034). Groups 2 and 3 do not differ significantly either ($p = 0.865$. SE 0.034).

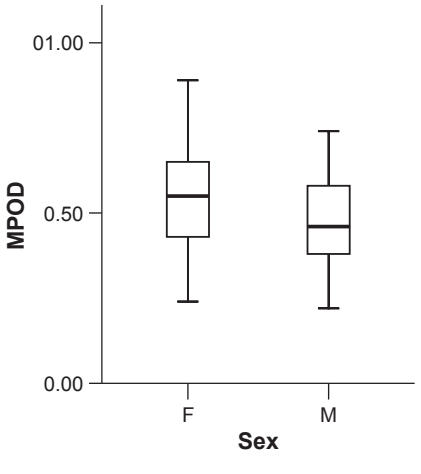

Fig. 2. Boxplots of mean macular pigment optical density (MPOD) for gender. Mean MPOD-Females 0.55 (CI 95% = 0.51–0.60) Mean MPOD-Males = 0.49 (CI 95% = 0.45–0.52). A slight but significant difference noted (difference in MPOD 0.059, 95% CI 0.005–0.112, $p = 0.031$).

disease in the fellow eye would be very helpful for rapid intervention. A cut-off of MP measurement for AMD progression would be very appealing as we could start treatment early enough and slow down the decrease in visual acuity in their remaining eye. Nevertheless, our findings did not prove our primary hypothesis. The MPOD of the fellow eye with dry AMD of patients with unilateral exudative AMD was found to be significantly *higher* compared with the MPOD of patients with the bilateral dry form of the disease. Moreover, the patients of our study group had higher level of MPOD compared with age-matched subjects with no AMD. However, this difference did not reach statistical significance when the factors age and gender were included in the regression model. Eventually, no significant difference was reported between bilateral dry AMD patients and healthy elderly control subjects.

The MPOD in the fellow eyes of patients with unilateral wet AMD was not found to be lower compared with individuals of similar age range and healthy retinas. Furthermore, we found higher levels of MPOD in the eye with dry AMD of these patients when compared with patients with bilateral dry AMD. Similar findings are scarce. To our knowledge, only Beatty et al. 2001 measured MPOD in nine high-risk patients with neovascular AMD in one eye and a healthy fellow eye. They tried to correlate MPOD with the risk factors for AMD, age and advanced disease in one eye. MPOD measurements were performed with HFP in 46 subjects and factors such as iris colour, smoking and gender were taken into consideration. Apart from the rest of their findings, they concluded that 8 of the 9 subjects with neovascular AMD in one eye had significantly less MPOD in their fellow eye compared with their age-matched control eyes (0.147 versus 0.311, $p = 0.015$). A negative correlation with age was also noted (right eye: $r^2 = 0.24$, $p = 0.0006$; left eye: $r^2 = 0.29$, $p = 0.0001$). However, the authors did not support that there is a critical value below which AMD is possible to develop. They suggested that this reduction might possibly reflect a response to an age-related process, such as oxidative stress. Besides, even in that small sample, there were patients that had higher levels than their low-risk normal match. Even if no age effect was detected in our study, the high levels of MPOD of our patients enhance the opinion that no such borderline value of MPOD exists.

Mechanisms related to wet AMD pathophysiology may offer a possible explanation for our findings, assuming that our measurements reflect exclusively the concentration of lutein and zeaxanthin at the central 1° of the macula. As it is known, AMD is a bilateral, most often asymmetrical, disease; it may be the case that the mechanism that leads to conversion from dry to wet AMD is accompanied with disturbances at the accumulation of several molecules, proteins and substances at the macular level, including retinal carotenoids. This could result in increased values of MPOD. Friedman et al. 1995 investigated the vasculature of eyes with AMD and discovered significant changes in both the retinal and choroidal circulation of these eyes. Over the years, many studies have confirmed these findings (Zhao et al. 1995; Grunwald et al. 2005; Pournaras et al. 2006). Alterations in circulatory status of eyes with advanced dry and wet AMD may affect the transport of lutein and zeaxanthin, increasing eventually their levels. Besides, it has been indicated recently that the dry AMD retinas can accumulate the xanthophylls to the same extend with the eyes with no retinal pathology (Koh et al. 2004).

Patients with bilateral dry AMD did not show any significantly lower levels of MPOD when compared with healthy subjects of the same age. This finding agrees with other studies that do not find any association between MPOD and progression of dry AMD. Berendschot et al. did not find in 2002 any difference in MPOD, measured with spectral fundus reflectometry, between normal and early AMD eyes of 435 subjects older than 55 years old. Similarly, in 2008, the CAREDS (carotenoids in Age-Related Eye Disease Study) Group did not find any cross-sectional association between MPOD and AMD when measured psychophysically (HFP) in 1698 subjects (54–86 years old). Kanis et al. (2007) reported that MPOD did not have any protective role in the progression of AMD in a group of 435 patients. We need to mention, though, that our sample size was not big enough to highlight small differences

and that the stage of the disease may affect the findings.

No age effect on MPOD levels was observed in the present study, in accordance with many previous studies. Berendschot et al. 2002 did not find any decrease in MPOD with age in 435 individuals when measuring with a fundus reflectance technique. Moreover, Ciulla & Hammond (2004) did not observe any effect of age on MPOD levels in 390 elderly subjects (mean age 72, SD = 8) measured with HFP, not even if they had cataract or age-related maculopathy. Iannaccone et al. (2007) did not find an age-related decline at the MPOD of the population of the ARMA study (222 normal elderly individuals), using an HFP technique. In addition, Berendschot & van Norren (2004) have used and compared five different measuring techniques to assess the MPOD in a healthy population of a wide age range (134 individuals, 19–76 years old); no evidence of any age effect on MPOD was provided, except for the HFP method (a slight age-decline of MPOD was showed), which was attributed to an increase in the parafoveal values and not an overall decrease. On the other hand, many other studies have found an age-related decrease in MPOD (Hammond et al. 2000; Beatty et al. 2001; Nolan et al. 2010). Nolan et al. (2007) found a decline of MPOD with age in an Irish population of 828 individuals (mean age: 42, SD = 12) as well as in a recent study (2010) of a smaller Irish sample [79 subjects, 65 (±11) years]. In the present study, the effect of age on MPOD was not apparent.

A small but statistically significant difference emerged between genders. The women of our study appear to have more MPOD than men. This finding contradicts other studies that indicate lower levels of MPOD in women than in men (Hammond et al. 1996a,b; Hammond & Caruso-Avery 2000). Nevertheless, many investigators find no differences between sexes (Berendschot et al. 2002; Iannaccone et al. 2007; Kanis et al. 2007), while Curran-Celentano et al. (2001), when measuring 280 subjects (17–50 years old) with an HFP method, found women to outmatch men in terms of MPOD levels. The sample size of the present study is restrictive for further conclusions.

In total, the mean MPOD as measured in this aged population of AMD patients and healthy elderly individuals is remarkably high (0.52, SD 0.15) when compared with values published by other investigators measuring with similar techniques (HFP). Hammond & Caruso-Avery (2000) measured in 217 subjects (mean age: 41.5 ± 19.7) a mean MPOD equal to 0.22(±0.13). Moreover, Ciulla & Hammond (2004) measured (with HFP) a mean MPOD equal to 0.26(±0.19) in 390 subjects of 18–88 years old, while Iannaccone et al. published in 2007 data from 222 elderly subjects (mean age:79.1, SD = 3.2) that reported a mean MPOD of 0.34 (±0.21). A reasonable cofactor to these high values could be the Mediterranean diet that is followed by the local population (study conducted in Crete) which is rich in green vegetables and fruits [sources of lutein and zeaxanthin (Sommerburg et al. 1998)]; even if retinal pigment epithelium changes characterizing the disease (Friedman 1997) might hinder the circulation of nutrients (including the carotenoids) to the retina, it has been indicated by Koh et al. (2004) that the eyes with dry AMD can accumulate lutein as effectively as the eyes with no retinal pathology. Thus, a diet rich in xanthophylls such as the Mediterranean diet could result in high levels of MPOD both in normal individuals and in individuals with AMD. Furthermore, the small percentage of smokers (especially among women) in our sample may have contributed to the high levels of MPOD (Hammond et al. 1996a,b). On the other hand, ethnic (Wolf-Schnurrbusch et al. 2007) and lifestyle characteristics such as increased body fat (Hammond et al. 2002; Nolan et al. 2004) and extended exposure to sunlight (West et al. 1989; Taylor et al. 1992; Darzins et al. 1997) may also have some effect on MPOD. Genetic factors also have been shown to play a role in MP concentration as Liew et al. 2005. A future study comparing the population of our study with age-matched populations with different hereditary and geographic characteristics would be interesting.

In this study, the MPOD was measured with heterochromatic flicker photometry, a psychophysical method broadly used for MPOD assessment because of its rapid and easy technique (Wooten et al. 1999; Snodderly et al. 2004; LaRowe et al. 2008; Nolan et al. 2007; Stringham et al. 2008). However, HFP has several drawbacks regarding mainly the compliance of the subject and the measurements in patients with low visual acuity. MPOD can also be measured with physical techniques such as fundus reflectometry (Berendschot & van Norren 2004, 2005) and autofluorescence (Delori et al. 2001; Delori 2004). These methods may be more objective, but they demand expensive equipment and procedures that are time-consuming and difficult for both the examiner and the examinee. Despite all efforts, no golden standard for quantifying the macular pigment exists (Hammond et al. 2005; Beatty et al. 2008; Leung & Phil 2008). The device that we used in our study has all the advantages and disadvantages of HFP techniques; one of its main advantages is related to its novel methodology for threshold determination that makes the test even quicker and easier. The strong correlation with the objective method of fundus reflectometry (Van der Veen et al. 2009) enhances the reliability of its results.

In conclusion, in the current study, the primary hypothesis that patients with wet AMD in one eye would have low levels of MPOD in their fellow eye was not proved. On the contrary, we found higher levels of MPOD in the eye with dry AMD of these patients when compared with patients with bilateral dry AMD and with no AMD age-matched subjects. Further investigation into the factors that affect the MPOD and its assessment as well as the pathophysiology of the disease and its progression is needed to evaluate the possible role of macular pigment in AMD.

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