Retina

Spatial Distribution of Macular Pigment in an Elderly French Population: The Montrachet Study

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Purpose. We describe the spatial distribution and cross-sectional associations with physical and lifestyle attributes.

Methods. The participants of the Montrachet (Maculopathy Optic Nerve, nuTRition neurovAsCular and HEarT) study underwent an exhaustive ophthalmologic examination, including optical coherence tomography, to measure retinal thickness. The spatial distribution of macular pigment optical density (MPOD) defined as a ring-like, intermediate or no-ring pattern, was assessed by means of the two-wavelength autofluorescence technique. Blood samples were taken to measure plasma lutein and zeaxanthin using high performance liquid chromatography (HPLC).

Results. We studied 635 subjects with good-quality MPOD measurements. The mean age was 82.0 ± 3.4 years. A ring-like structure was found in 109 (17.2%) participants. Participants with a ring-like spatial distribution had a thinner macula in the central subfield ($P < 0.001$). In multivariate analysis, individuals never protecting themselves from sunlight and with dark iris color were less likely to present a ring-like than a no-ring spatial distribution ($P = 0.033$ and $P = 0.013$, respectively). Only in males were lutein plasma levels higher in ring-like and intermediate MPOD profiles than in no-ring structures (odds ratio [OR] = 1.59 for 100 additional μg; 95% confidence interval [CI] = 1.16–2.18 and OR = 1.64; 95% CI, 1.17–2.28).

Conclusions. In this population-based study, individuals never protecting themselves from sunlight, and with dark iris color and a thinner macula were less prone to displaying a ring-like MPOD spatial distribution.

Keywords: macular pigment optical density, ring-like structure, lutein, zeaxanthin, elderly, population-based study

Macular pigment (MP) is composed of three carotenoids, two of dietary origin, lutein (L) and zeaxanthin (Z), and meso-Z synthetized from L.[1] The MP is located in the inner layers of the retina, the highest concentration being in the Henle fiber layer of the fovea and rapidly decreasing to become undetectable outside the macula.[2] Zeaxanthin prevails in the central fovea, whereas L still can be detected at 8° eccentricity.[3]

The key role of MP is to protect the macula from high-energy light wavelengths[4] and to buffer free radicals generated by the oxidative processes of visual transduction.[5] More recently it was shown that MP has other positive effects on the retina.[1] It also can improve vision by reducing glare and increasing contrast sensitivity.[6–10]

Several techniques are available to measure MP with macular pigment optical density (MPOD); they are varied and share certain advantages and disadvantages.[11] Macular pigment optical density is expressed as a quantitative variable with a rapid decline from the center of the fovea to 6° eccentricity. In addition, the qualitative aspect of MPOD distribution has been reported. Berendschot and van Norren[12] and Delori et al.[13] were the first to describe a bimodal distribution of MP or a ring-like structure with a central peak surrounded by a secondary peak. It has been shown that the ring-like profile was more frequent in African subjects[14] and women.[13] Moreover, the ring-like structure was associated with a lower risk of age-related macular degeneration (AMD) in the Muenster Aging and Retina Study (MARS).[15] Two other MPOD spatial distributions have been reported. The intermediate pattern indicates that no ring-like profile is visible and that the decline of MPOD from the center of the fovea to the periphery is not monotonic, showing a plateau on the MPOD profile.[15] The no-ring structure corresponds to a monotonous decline of MPOD without any ring-like or plateauing pattern.[15] The spatial distribution of MPOD seems resistant to oral carotenoid supplementation, possibly indicating that these MPOD profiles are mainly driven by individual and/or environmental factors.[16] Recently, macular architecture and thickness have been investigated and it was shown that higher MPOD and ring-like structures were more frequent in thinner maculae.[17]

Investigative Ophthalmology & Visual Science

Macular pigment optical density spatial distribution and its relation with risk factors of AMD and with plasma carotenoids are not clearly established in the elderly. Therefore, we conducted a study to describe MPOD spatial distribution and its associations with individual and environmental factors in a population-based study, the Maculopathy Optic Nerve and nuTRition neurovAsCular and HEarT (Montrachet) study, conducted in a population older than 75 years.

Patients and Methods

Population Study

The Montrachet study is an ancillary study of the population-based Three Cities (3C) study, which already has been reported.[18] The 3C cohort study was undertaken to assess the relationships between vascular risk factors and aging disorders. Overall, 9294 persons aged 65 years and over, selected from the electoral rolls of three French urban cities (Bordeaux, Dijon, and Montpellier), were included ($n = 4931$ living in Dijon). Ten years later, a subgroup of participants from Dijon were asked to participate in the Montrachet study. The Montrachet study was designed to assess associations between age-related eye diseases, and neurologic and heart diseases in the elderly. The methodology of the Montrachet study and the baseline characteristics of volunteers have been published recently.[19] Briefly, from October 2009 to March 2013, 1153 volunteers underwent a comprehensive eye examination in the Department of Ophthalmology of the Dijon University Hospital (France). This examination included the collection of self-reported eye diseases and treatment history, visual acuity measurement, refractive error identification, IOP measurement, visual field examination, optical coherence tomography (OCT) imaging, retinal photographs, and MPOD measurement in participants with exploitable macula images. Fasting blood samples were drawn to measure plasma carotenoids and fatty acids. Finally, all participants were asked to complete a questionnaire on lifestyle (alcohol consumption and smoking status), environment (sun protection), and nutrition (food frequency questionnaire). The consumption of oral supplements containing xanthophylls was documented on self-declaration. Written informed consent was obtained from all participants. The study followed the tenets of the Declaration of Helsinki and was approved by the regional ethics committee (Number 2009-A00448-49).

MPOD Measurements

Macular pigment optical density was measured with the two-wavelength autofluorescence method using the modified Heidelberg Retina Angiograph (HRA; Heidelberg Engineering Co., Heidelberg, Germany).[20,21] After pupil dilation and retinal bleaching, sequences of 20° images at 30-second intervals were captured and two acquisitions were made at 488 and 514 nm.[13,22] For the study, MPOD values given in optical density units (DU) at 0.5°, 1°, 2°, and 6° eccentricity were recorded. Both eyes were evaluated for MPOD with a high correlation between the two eyes ($r = 0.93$).[22] The eye with the best image quality was retained for analysis. The right eye was chosen when image quality was similar in both eyes. We excluded participants with poor image quality in both eyes and those who suffered from late AMD, considering that geographic atrophy or neovascularization affects the measurement and spatial profile of MPOD.[23] The spatial profile of MPOD was determined by a trained investigator as previously described in the literature.[15,17] From the graphs generated by the software of the modified HRA, a ring-like profile was defined by a central peak followed by a decline and then a second peak on the radial density profile (Fig. 1A).[15] An intermediate profile corresponded to a nonmonotonic decrease of the MPOD graph without a bimodal pattern (Fig. 1B). A no-ring profile was defined as a no-bimodal pattern and a monotonous decline of the MPOD graph (Fig. 1C). To evaluate the reliability of this classification, a second investigator analyzed 100 randomly selected eyes of the cohort in a masked manner. The agreement for spatial profiles of MPOD between the two investigators was good with a κ value of 0.71 (95% confidence interval [CI] = 0.48–0.93).

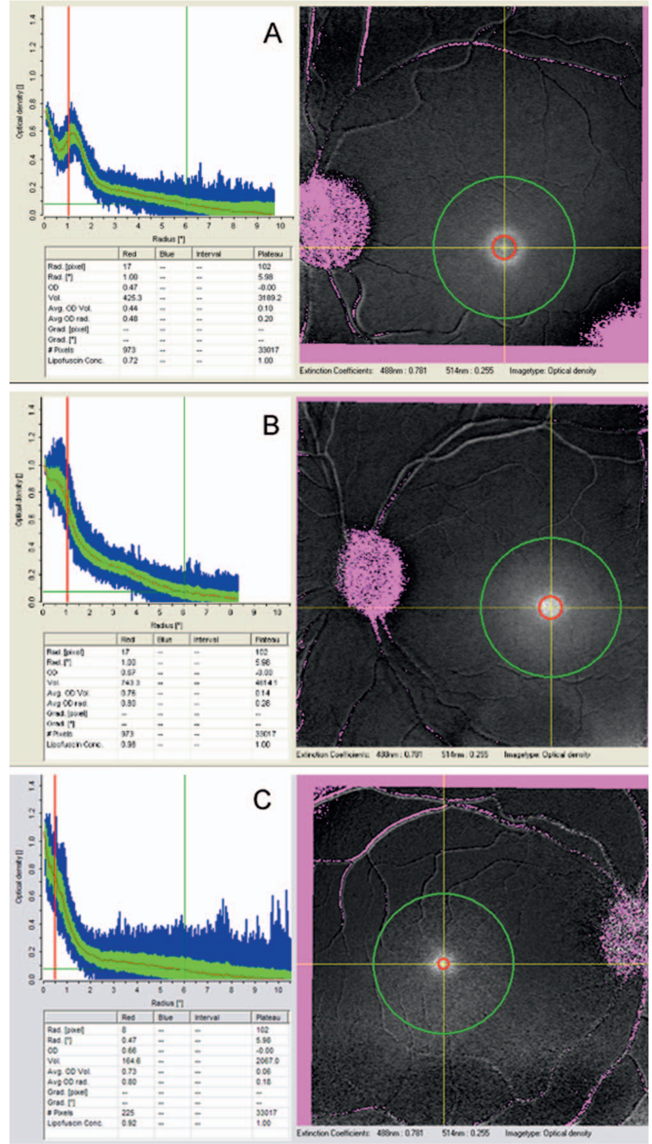

Figure 1. The three patterns of MPOD spatial distribution as obtained with the dual-wavelength technique. **(A)** ring-like profile, **(B)** intermediate profile, **(C)** no-ring profile.

Fundus Photographs and OCT Imaging

Technicians took the retinal photographs with a fundus camera (TRC NW6S; Topcon, Tokyo, Japan) and macular OCT was performed with the Spectralis SD-OCT (Heidelberg Engineering) after pupil dilatation with one drop of tropicamide 2 mg/0.4 mL. The OCT acquisition had a pattern size of 20° × 15° with 19 B-scans. Two trained ophthalmologists classified the eyes into early AMD stage 0–3 and late AMD as stage 4 according to the Alienor study, another French population-based study conducted in the elderly.[24] Macular thickness was noted for each subfield of the Early Treatment Diabetic Retinopathy Study (ETDRS) map on the OCT layout.[25] In this study, we retained for analysis only the central subfield value (CSF) representing the average of all points within the inner circle within a 1-mm radius.[26]

Blood Dosage

Blood samples were drawn from fasted subjects in our department and stored at −80°C before analysis, as reported previously.[22] Briefly, plasma fatty acids were analyzed by gas chromatography, as described previously.[27] Lutein and Z plasma levels were measured using high-performance liquid chromatography (HPLC). After extraction with absolute ethanol and hexane, we used two HPLC columns in tandem (Nucleosil C18, 25 × 4.6 mm ID, 5 μm; Thermo Finnigan, Villebon-sur-Yvette, France, and VIDAK C18, 25× 4.6 mm ID, 5 μm; Altech France, Epernon, France) for measurements. The analytes were identified by their absorption spectra and their retention times.[22]

Statistical Analysis

Continuous variables were expressed using mean (SD) or median (interquartile range [IQR]), whether or not they had a normal distribution according to the Kolmogorov-Smirnov test. Qualitative variables were described using frequencies. Continuous variables were compared using Student *t*-tests, ANOVA, Mann-Whitney, or Kruskal-Wallis tests when appropriate. Frequencies were compared using χ^2 tests or Fisher exact tests. Polytomous logistic regression models were used to estimate the association between participants' characteristics as well as plasma levels of (1) L and (2) Z with the MPOD spatial profile (ring-like, intermediate pattern versus no-ring pattern) while accounting for the factors associated with the MPOD spatial profile. All variables associated with the MPOD spatial pattern in bivariate analyses with a P value < 0.2 were included in the model. A backward procedure was applied to obtain the final models. In the second step, MPOD at 0.5° and 2° was included in the final model to estimate their association with the MP profile while accounting for confounders. P values below 0.05 were considered statistically significant. Data analyses were performed using SAS software (version 9.3; SAS Institute, Inc., Cary, NC, USA).

Results

Among the 1153 participants of the Montrachet study, 635 subjects had MPOD data suitable for analysis (Fig. 2). Compared to nonparticipants, participants in this study were more likely to be pseudophakic, had earlier AMD stages, and marginally displayed dark brown irides (See Supplementary Table S1). The mean age was 82.0 ± 3.4 years and 61.3% were women. Overall, a ring-like profile was observed in 109 subjects (17.2%), an intermediate pattern in 67 (10.5%), and a no-ring structure in 459 (72.3%). In the ring-like profile, the second peak had an average eccentricity of 0.84° ± 0.16°.

We tested the symmetry between the right and left eyes for macular pigment distribution in 100 randomly selected participants and we found good agreement between the two eyes ($\kappa = 0.79$, 95% CI = 0.64–0.95). The results of bivariate analysis are given in Table 1. Subjects with a ring-like or intermediate profile had a thinner macula than those with no ring ($P < 0.001$). Women had a significantly more ring-like spatial distribution than men ($P = 0.034$). Macular pigment optical density was significantly lower at 0.5° eccentricity in the ring-like profile ($P = 0.002$) and higher at 2° and 6° eccentricity ($P = 0.001$ and $P = 0.002$, respectively; Table 1). Macular pigment optical density spatial distribution was not associated with sun protection, alcohol consumption, smoking statusm or the presence of early AMD.

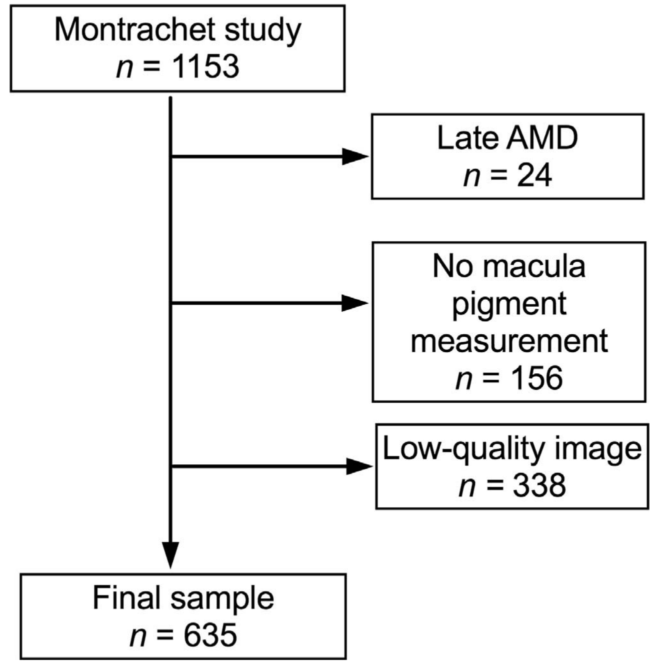

Figure 2. Flowchart of the Montrachet study and the MPOD distribution study.

Multivariate analysis showed that individuals never protecting themselves against sun had a ring-like pattern less frequently than a no-ring profile. Individuals with dark iris color had a ring-like pattern less frequently than a no-ring pattern compared to individuals with an iris of a lighter color (odds ratio [OR] = 0.46; 95% CI, 0.25–0.85; Table 2). Subjects with a thicker macula were less prone to have a ring-like or an intermediate pattern than those with a no-ring pattern (OR = 0.97; 95% CI, 0.96–0.98 and OR = 0.98; 95% CI, 0.97–0.99, respectively). These results did not differ when adjusted for MPOD at 0.5° and then 2° eccentricity. The higher MPOD level at 0.5° was associated with a lower occurrence of the ring-like pattern ($P = 0.003$) (See Supplementary Table S2). Conversely, higher MPOD at 2° was associated more frequently with an intermediate and ring-like pattern (See Supplementary Table S2).

Lutein and Z plasma dosages were available in 441 participants. This subgroup displayed the same clinical characteristics as the participants without plasma samples (See Supplementary Table S3). Median plasma L and Z levels were 283.8 (179.6–460.7) μg/L and 17.6 (11.7–26.5) μg/L, respectively. In bivariate analysis, subjects with a ring-like profile had significantly higher plasma L and Z levels than the other types of MP profiles ($P = 0.009$ and $P = 0.014$, respectively; Table 1). When estimating the adjusted association between L and the MPOD profile using a multivariable polytomous logistic regression, we found a significant interaction between L plasma concentration and sex (Table 3). Indeed, in men, the higher the L plasma level, the higher the frequency of an intermediate (OR for 100 additional μg/L = 1.64; 95% CI = 1.17–2.28) or a ring-like pattern (OR = 1.59; 95% CI = 1.16–2.18) compared to a no-ring profile. Conversely, the L plasma level was not associated with MPOD profiles in

Table 1. Characteristics of Participants in the Evaluation of Macular Pigment Distribution in the Montrachet Study, Bivariate Analysis

	No Ring, *n* = 459	Intermediate, *n* = 67	Ring-Like, *n* = 109	*P* Value
Age, y				
<=80	162 (35.3)	22 (32.8)	46 (42.2)	0.515
80–85	196 (42.7)	33 (49.3)	41 (37.6)	
>85	101 (22.0)	12 (17.9)	22 (20.2)	
Sex				
Male	191 (41.6)	24 (35.8)	31 (28.4)	**0.034**
Female	268 (58.4)	43 (64.2)	78 (71.6)	
Smoking status*				
Never	283 (63.0)	43 (64.2)	79 (73.2)	0.145
Current or past	166 (37.0)	24 (35.8)	29 (28.9)	
Alcohol consumption				
Never	125 (27.2)	23 (34.3)	35 (32.1)	0.338
Current or past	334 (72.8)	44 (65.7)	74 (67.9)	
BMI, kg/m^2				
<25	192 (41.8)	32 (47.8)	54 (49.5)	0.272
≥25	267 (58.2)	35 (52.2)	55 (50.5)	
Sun protection*				
Never	53 (11.6)	6 (9.0)	6 (5.5)	0.127
Occasionally	95 (20.8)	19 (28.4)	19 (17.4)	
Often	309 (67.6)	42 (62.7)	84 (77.1)	
Lens status				
Phakic	215 (46.8)	29 (43.3)	44 (40.4)	0.441
Pseudophakic	244 (53.2)	38 (56.7)	65 (59.6)	
Iris color				
Blue/grey	179 (39.0)	24 (35.8)	52 (47.7)	**0.033**
Green/hazel	147 (32.0)	29 (43.3)	39 (35.8)	
Brown/black	133 (29.0)	14 (20.9)	18 (16.5)	
Central retinal thickness, μm*	290.0 [274.0–311.0]	274.0 [262.0–294.0]	270.0 [253.0–287.0]	**<0.001**
Early AMD stage*				
0	236 (54.3)	38 (59.4)	61 (58.1)	0.753
1	154 (35.4)	22 (34.4)	38 (36.2)	
2	36 (8.3)	4 (6.3)	6 (5.7)	
3	9 (2.1)	0 (0.0)	0 (0.0)	
LZ supplementation				
No	432 (94.1)	65 (97.0)	104 (95.4)	0.683
Yes	27 (5.9)	2 (3.0)	5 (4.6)	
MPOD at 0.5°, DU	0.58 ± 0.27	0.56 ± 0.21	0.48 ± 0.24	**0.002**
MPOD at 1°, DU	0.47 ± 0.22	0.51 ± 0.18	0.48 ± 0.22	0.355
MPOD at 2°, DU	0.29 ± 0.14	0.34 ± 0.13	0.34 ± 0.15	**0.001**
MPOD at 6°, DU	0.07 ± 0.04	0.08 ± 0.04	0.09 ± 0.04	**0.002**
L, μg/L†	263.4 (173.4–431.9)	320.9 (178.8–487.9)	366.6 (221.0–492.2)	**0.009**
Z, μg/L†	16.1 (11.0–25.5)	18.9 (12.9–27.5)	21.2 (14.4–29.5)	**0.014**

Bold *P* values indicate a statistically significant difference at the threshold of 5%. The results are displayed as *n* (%) for categorical variables and mean ± SD or median (interquartile range) for continuous variables depending on their distribution.

* Missing data for smoking status ($n=11$), sun protection ($n=2$), central retinal thickness ($n=2$), AMD stage ($n=31$), L/Z supplementation ($n=34$).

† L and Z plasma levels were only available for 441 participants (no ring = 315, intermediate = 48, ring-like = 78).

women ($P = 0.580$ for ring-like versus no ring like pattern, $P = 0.716$ for intermediate versus no ring-like pattern). No significant association was found for Z (data not shown).

Discussion

The ring-like structure of MPOD was first reported a decade ago.[12,13] Since then, several studies have tried to improve our knowledge on MP spatial distribution using different techniques, either with autofluorescence images[14,15] or heterochromatic flicker photometry.[2,28] In the present study, we found 17.2% ring-like patterns, that is, similar to another study investigating the same age range, 19.8% for example in the MARS study.[15] The secondary peak measured at 0.84° eccentricity also was in accordance with the literature, 0.85° in the MARS study[15] and other reports ranging from 0.66° to 0.70°.[12–14] However, previous studies examining younger healthy individuals described ring-like patterns in over half of the cases[12,13] and up to 68% in non-Hispanic white subjects.[14] It has been hypothesized that the ring-like structure could be subjected to change in an individual's lifetime, but this remains

Table 2. Associations of Physical and Lifestyle Characteristics of Montrachet Participants With Macular Pigment Distribution

	MPOD Profile			
	Intermediate		Ring-Like	
Versus No-Ring Pattern	OR (95% CI)	P Value	OR (95% CI)	P Value
Sun protection				
Never vs. often	0.73 (0.29–1.82)	0.495	0.37 (0.15–0.92)	**0.033**
Occasionally vs. often	1.30 (0.71–2.38)	0.382	2.68 (1.08–6.64)	0.071
Iris color				
Green vs. blue	1.44 (0.79–2.60)	0.224	0.88 (0.53–1.45)	0.619
Brown vs. blue	0.79 (0.39–1.60)	0.523	0.46 (0.25–0.85)	**0.013**
Central retinal thickness, μm	0.98 (0.97–0.99)	**0.007**	0.97 (0.96–0.98)	**<0.0001**

Bold *P* values indicate a statistically significant difference at the threshold of 5%. A total of 622 observations were used due to missing values for sun protection and central retinal thickness.

to be firmly established.[15] In the literature, the ring-like structure was more frequent in females and never smokers, while this MP pattern was less frequent in eyes with early AMD stages.[15] In our study, even though an association between the ring-like pattern and female sex was observed in bivariate analysis, this was no longer the case in multivariate analysis. Moreover, we did not find an association between the MP profile and smoking status nor the AMD stage or body mass index (BMI), as was shown in one study.[29] This difference could originate from the different designs of the MARS study, a longitudinal case–control study, and the Montrachet population-based study. The first results with the Montrachet population have shown that these participants are overall in good health, with a low number of subjects suffering from dementia or low visual acuity and with a low AMD rate.[19,22] Therefore, the low number of AMD cases and missing data related to poor fundus photographs could have weakened a potential association between AMD with different variables.

In multivariate analysis, we have found that participants with a MPOD ring-like spatial distribution had a negative association between protection from sunlight, dark iris color, and macular thickness. Controversial reports have addressed sunlight exposure and light iris color as potential risk factors for developing AMD.[30–32] Interestingly, a recent study investigated the risk for AMD and sun exposure. The authors found that past sunlight exposure was associated with early AMD but not current exposure.[33] Moreover, these authors did not find any association between iris color and AMD. Although the populations differ in age and ethnicity, it is worth mentioning that the ring-like pattern was more common in Africans when compared to non-Hispanic whites.[14] Nevertheless, the hypothetical prevention of light damage by iris color and how it could influence MP distribution is poorly documented in the literature and deserves further elucidation.

In our population, eyes with a ring-like structure had a thinner macular thickness, as indicated by the automatic measurements of the central subfield of the EDTRS chart. This finding remained in multivariate analysis for intermediate and ring-like MPOD profiles. This association is in accordance with a recent publication, although the measurement of macular thickness was different and more refined in evaluating the foveal pit slopes in this study.[17] Indeed, the foveal anatomy could influence the spatial distribution of MP,[2] and it has been suggested that a ring-like distribution is associated with thinner inner retinal layers.[17] The association between higher MPOD at 2° and the ring-like profile found in our study is in line with the results of Huntjens et al.,[34] although ethnicity and the method for MP measurement were different from our study.

In a subgroup of 441 participants, we focused on L and Z plasma concentrations and investigated the relation of these

Table 3. Associations of Sun Protection, Central Retinal Thickness, and Plasma L With Macular Pigment Distribution According to Sex (Montrachet Study; Multivariate Analyses)

	Macular Pigment Distribution			
	Intermediate vs. No Ring		Ring-like vs. No Ring	
	OR (95% CI)	P Value	OR (95% CI)	P Value
Males				
Sun protection				
Never vs. occasionally	0.72 (0.10–4.92)	0.738	0.64 (0.12–3.39)	0.602
Often vs. occasionally	2.58 (0.72–9.23)	0.144	0.82 (0.91–3.59)	0.677
Central retinal thickness, μm	0.99 (0.98–1.01)	0.473	0.96 (0.94–0.98)	**0.0007**
L, 100 μg/L	1.29 (1.09–1.52)	**0.002**	1.22 (1.05–1.43)	**0.009**
Females				
Sun protection				
Never vs. occasionally	1.12 (0.34–3.67)	0.849	0.21 (0.05–0.96)	**0.045**
Often vs. occasionally	2.16 (0.90–5.17)	0.082	1.16 (0.55–2.43)	0.638
Central retinal thickness, μm	0.99 (0.98–1.00)	0.142	0.98 (0.97–0.99)	**0.005**
L, 100 μg/L	0.96 (0.83–1.10)	0.580	1.01 (0.92–1.12)	0.716

Bold *P* values indicate a statistically significant difference at the threshold of 5%. L plasma levels were only available for 441 participants (no ring = 315, intermediate = 48, ring-like = 78). A total of 440 observations were used due to missing values for sun protection.

carotenoids and the spatial distribution of MP. Median plasma L and Z levels were 283.8 and 17.6 ng/mL, which is higher than in some recently published series.[35,36] In multivariate analysis, higher L plasma concentration was associated with intermediate and ring-like MP profiles only in males. No significant association was found for MP profiles in females as well for Z plasma concentration. Since this is the first study, to our knowledge, to take into account the plasma levels of the two main carotenoids in humans and the MPOD profile, any interpretation should be cautious. It should be noted that an interventional study in AMD patients has shown that carotenoid intake did not greatly modify the preexisting MPOD spatial profiles.[16] The high concordance of MPOD profiles between the two eyes[23] and the consistency of MPOD profiles after carotenoid supplementation led some authors to hypothesize that MPOD spatial distribution is mainly driven by the personal characteristics of each individual and possibly a genetic regulation.[15,37,38]

The strengths of the present study stem from the design of a population-based study and the availability of MPOD spatial profiles and plasma carotenoids in a large sample. Moreover, the results found herein for MPOD and macula thickness are in agreement with the literature.[15,16,26]

We acknowledge several limitations to this study. First, due to imaging quality and availability, we analyzed only 635 subjects of 1153 participants in the Montrachet study. The differences between participants and nonparticipants were focused on the lens status, AMD stages, and iris color. It has been shown that these 3 variables could affect the quantitative measurement of MPOD. We cannot exclude that not considering approximately 45% of our population could have impacted our results. Second, the interinvestigator agreement for classifying MPOD profiles was good, leaving room for misclassifications in some cases. Since to date no automatic algorithms to classify MPOD spatial distribution are available, these errors seem unavoidable. Third, the findings in this Caucasian, urban population, globally in good health, cannot be extrapolated to other age groups, ethnicities, or countries. Fourth, the estimation of sun protection, a surrogate for sun exposure used in other studies, is limited by self-reporting from the participants. Fifth, we were unable to show any relation between MPOD spatial distribution and AMD stages, which could be related to the general good health status and easy access to ophthalmologists of the urban Montrachet population, leading to a low AMD prevalence. Moreover, missing data due to poor-quality fundus photographs decreased the power of our analysis. Sixth, the cross-sectional nature of our study is a limitation per se, since past changes in lifestyle (sun protection, dietary habits) could affect MPOD and MP distribution.

In conclusion, in this elderly population, ring-like macular pigment spatial distribution was associated with sun protection, dark iris color, and a thinner macula, but not with other environmental factors. Lutein plasma levels were associated only with the intermediate and ring-like MPOD spatial distribution in males.

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