The Spatial Profile of Macular Pigments Is Related to the Topological Characteristics of the Foveal Avascular Zone

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Purpose. Macular pigments are preferentially concentrated in the central fovea, an area devoid of vasculature. We hypothesized that there may be a link between the macular pigment profile and the size and structural characteristics of the foveal avascular zone (FAZ).

Methods. Two-wavelength autofluorescence method was used to quantify macular pigment optical density (MPOD) and the radius at half peak of MPOD, which was defined as the retinal eccentricity where the MPOD value was 50% of the peak value. Volumetric spectral-domain optical coherence tomography (OCT) images of the macula were obtained from 32 subjects. The equivalent radius of the FAZ was determined using data generated from OCT angiography. Generalized estimating equations were used to test the hypothesis that there are interrelationships among the central foveal thickness, peak MPOD, the radius at half peak of MPOD and the equivalent radius of the FAZ.

Results. The equivalent radius of the FAZ was highly correlated with the radius at half peak of MPOD ($P < .001$). The equivalent radius of the FAZ was a significant predictor for central foveal thickness ($P < .001$). The significant predictor for peak MPOD was central foveal thickness ($P = .004$). Eyes with larger FAZs were more likely to have a secondary peak in their MPOD spatial profile in a zone ranging from 0.5 to 1.0 degrees from the foveal center.

Conclusions. The spatial distribution of macular pigment is related to the size of the FAZ, in addition to the central foveal thickness. It is possible that xanthophyll pigment accumulation in the macula serves functions, such as attenuation of shorter wavelengths of light, that would have been provided by the light-filtering characteristics of blood vessels.

Keywords: macular pigment, fovea, carotenoids, foveal avascular zone, optical coherence tomography

Macular pigments are concentrated in the central fovea and are preferentially deposited within the inner plexiform and Henle's fiber layers of the human macula.[1,2] The spatial profile of macular pigments, however, is not uniform and demonstrates significant interindividual variation, similar to foveal pit geometry and macular cyto-architecture.[3–6] It is unclear if the spatial profiles of macular pigments are correlated to the morphometric properties of the fovea by a range of anatomical interrelationships. Clarifying such relationships is important as it may aid our understanding about the functions served by macular pigments in retinal physiology and development.

The foveal avascular zone (FAZ) is an anatomic specialization that serves to maximize visual function by removing angioscotomas from the visual image at fixation. From an embryological standpoint, the FAZ is correlated to the development of the foveal pit.[7–9] Macular pigment is greatest in the center of the macula, which is devoid of vasculature.[10] Diseases with an absent or pathologically constricted FAZ, such as retinopathy of prematurity and albinism, are also known to have an absent or attenuated macular pigment optical density (MPOD) profile.[11] The relationship between foveal vascular topology and MPOD spatial profile has not been previously investigated in a quantitative manner. We hypothesized that foveal structure, as quantified by the size of the FAZ and the foveal thickness, may correlate with the distribution of macular pigment. The purpose of this study was to test the hypothesis that FAZ morphometry, MPOD spatial profile, and foveal architecture are interrelated in healthy subjects. This study provides new information concerning macular physiology and the associations between the FAZ and foveal metrics. Results from this study are used to speculate on the role of macular pigments in foveal structure-function relationships.

Materials and Methods

The study was approved by the Western Institutional Review Board. Informed written consent was obtained from subjects after explanation of the nature and possible consequences of the study. The protocol followed the tenets of the Declaration of Helsinki. The study was performed at Vitreous, Retina, Macula Consultants of New York, a private retinal clinic, from January to April 2015. The study examined healthy volunteer subjects who were friends or family members of the investigators of this study. All participating subjects were older

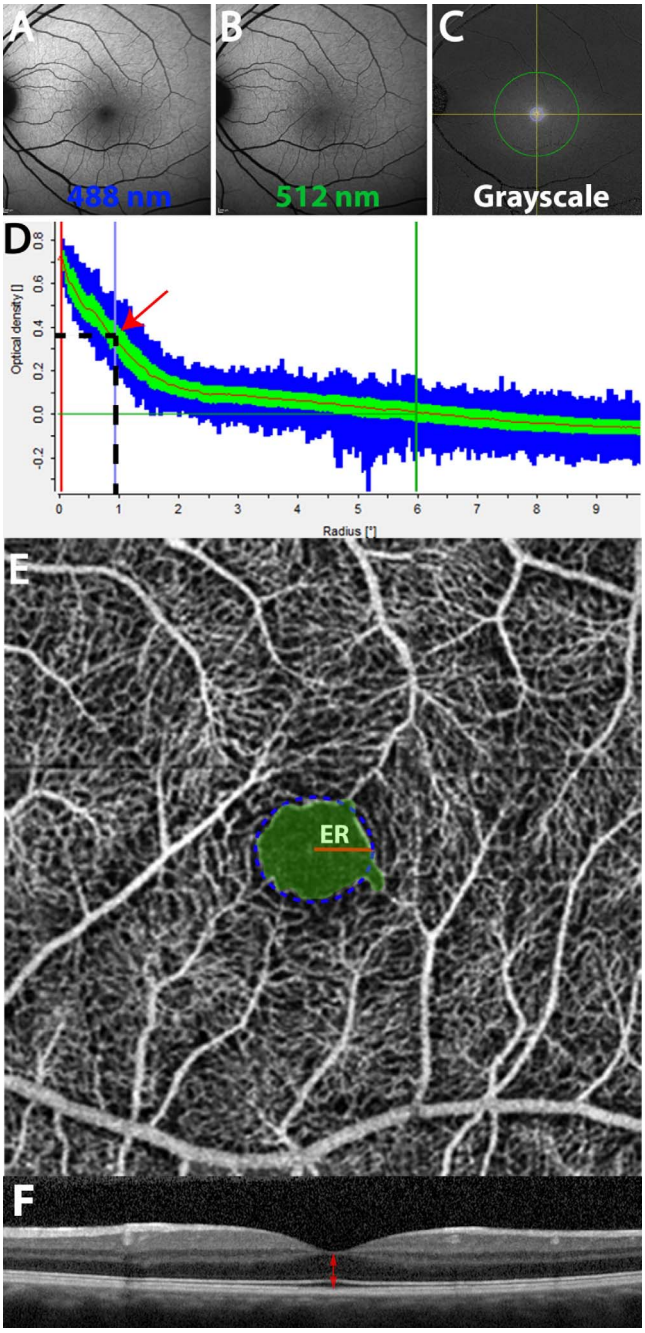

FIGURE 1. Quantitative analysis of MPOD spatial profile and FAZ size. The dual autofluorescence method for quantifying MPOD uses images excited by wavelengths that are differentially absorbed by macular pigment (488 nm [**A**], and 512 nm [**B**]). The differences in log intensity between these two images are used to generate a grayscale image (**C**) in which pixel intensity is proportional to MPOD. The spatial profile of MPOD (**D**) is generated by inbuilt software that compares MPOD values at the fovea to a reference point, which in this study was defined as the 6-degree eccentricity (*green lines*). Measurements were taken of Peak MPOD (*red vertical line*) and the eccentricity where the MPOD value was measured as 50% of the peak MPOD (*red arrow*; *fenestrated line*). The latter point was defined as the radius at half peak of MPOD. The FAZ size (**E**) was determined by calculating the area of the FAZ (*green shade*) on OCTA, approximating a circle to fit this area (*blue dashed line*) and calculating the radius of this circle (*red line*). This was defined as the equivalent radius (ER) of the FAZ. Structural OCT (**F**) was used to determine CFT (*double arrow*), which was defined as the distance from the RPE to the inner limiting membrane.

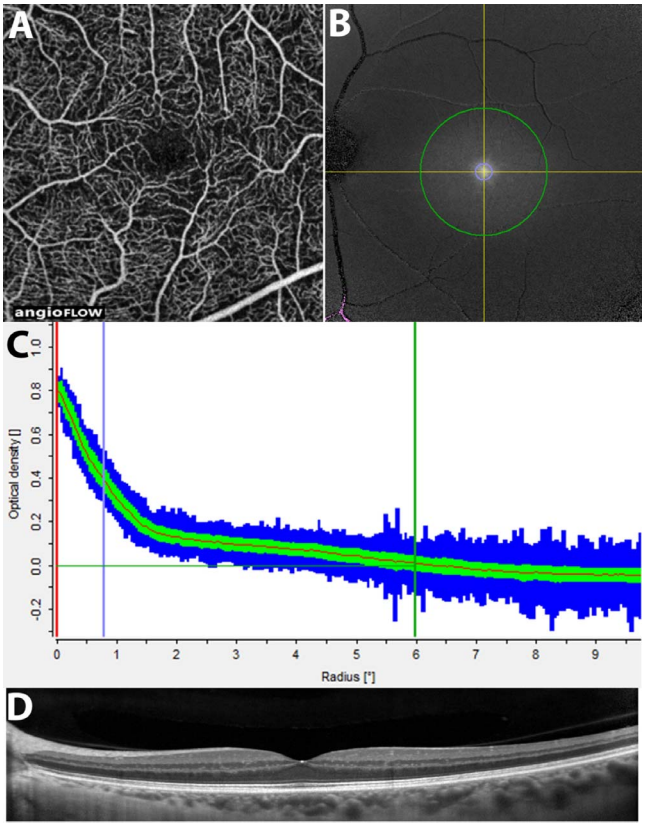

FIGURE 2. Topological relationships between the FAZ (**A**), MPOD spatial profile (**B**, **C**) and CFT (**D**) in an eye with a small FAZ. Note the monotonic decline in MPOD between the foveal center and peripheral eccentricities. The radius at half peak MPOD is approximately 0.75° (*vertical blue line*).

than 18 years and had no known history of ocular or systemic diseases. None of the subjects were using vitamin supplements. Data were acquired from both eyes of all subjects with the exception of five individuals who requested only one eye to be evaluated for this study.

Macular Pigment Optical Density Spatial Profile

The technique that was used to determine the MPOD spatial profile is illustrated in Figure 1. The MPOD measurements were determined by using a dual wavelength autofluorescence technique that has previously been described.[12] All measurements were obtained using the confocal scanning laser ophthalmoscope on the Heidelberg Retina Angiograph (Heidelberg Engineering, Inc., Heidelberg, Germany).[13,14] Once steady fixation was maintained, the scanning laser ophthalmoscope was focused on the macular region and autofluorescence images were captured at 488 nm and 512 nm within a 20° field centered at the fovea. System software was then used to generate an average MPOD grayscale map by digital subtraction of log autofluorescence images.[12] The intensity of the grayscale map (0–255 units) was proportional to MPOD. The MPOD spatial profile was generated from the grayscale map by calculating mean MPOD values for circles centered on the fovea. The reference location was selected at an eccentricity of 6° because previous reports have shown that the MPOD spatial profile flattens at approximately this location in healthy human subjects.[3,5,15,16] The radius at half peak of MPOD was also manually determined from the MPOD map and was defined as the retinal eccentricity (in degrees) where the

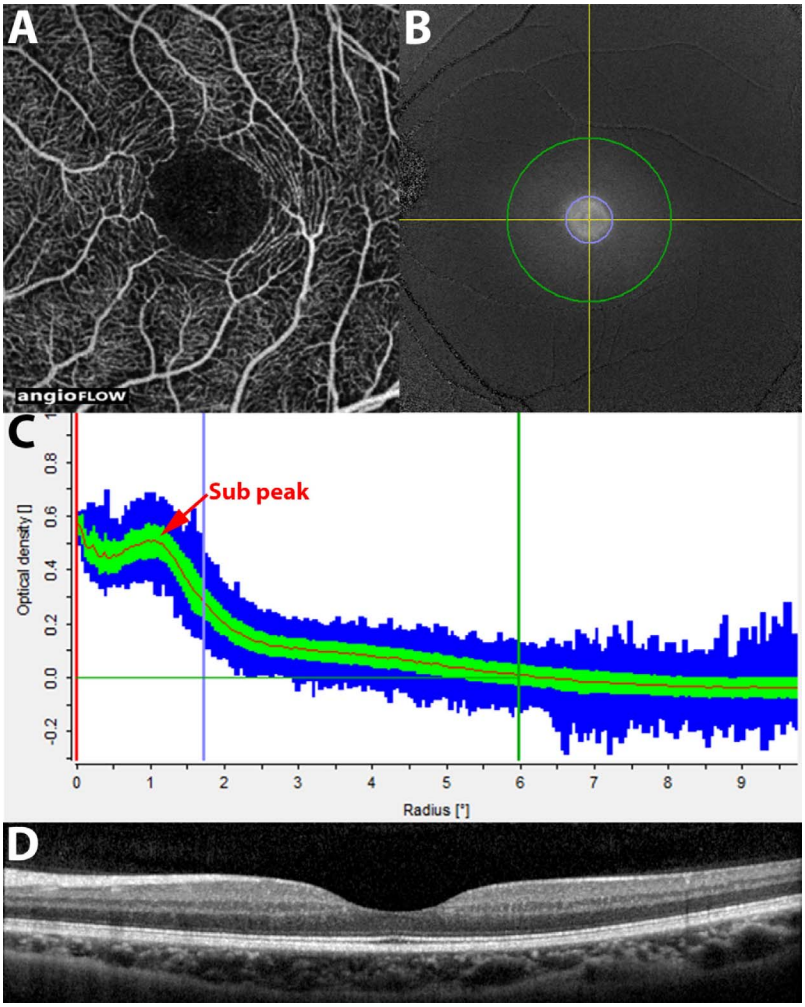

FIGURE 3. Topological relationships between the FAZ (**A**), MPOD spatial profile (**B**, **C**), and CFT (**D**) in an eye with a large FAZ. Note the subpeak in the MPOD spatial profile at approximately 1° eccentricity. The radius at half peak MPOD is approximately 1.7° (*vertical blue line*). On spectral-domain OCT, the central fovea appears thinner than eyes with small FAZs and this correlates with a lower peak MPOD value.

MPOD value was 50% of the peak MPOD. The radius at half peak of MPOD was determined by two independent examiners and the average measurement was used for statistical analysis.

Qualitative assessments of the MPOD spatial profile were also performed. If the decrease in MPOD approximated an exponential, monotonic decline with eccentricity, it was classified as a "no-ring profile" (Fig. 2). If subpeaks were observed in the MPOD spatial profile in a zone ranging from 0.5 to 1.0 degrees from the foveal center, then it was classified as a "ring profile" (Fig. 3).[17,18]

Central Foveal Thickness and Axial Length Measurements

Volumetric images of the central $20^0 \times 15^0$ were captured using high-resolution spectral-domain optical coherence tomography (OCT) on the Heidelberg Spectralis (Heidelberg Engineering). Volumes were composed of 49 B-scans with 9 A-scans averaged for each B-scan. The B-scan image of the central fovea was used for quantitative analysis. Point thickness of the central fovea was measured with the calipers provided by the OCT software and was defined as the distance between the RPE and inner limiting membrane (Fig. 1). Central foveal thickness (CFT) was measured by two examiners and the average measurement was used for statistical analysis. Axial length measurements were collected with the IOLMaster (Carl Zeiss Meditec, Dublin, CA, USA), which uses partial coherence interferometry to calculate the length of the eye.

Foveal Avascular Zone Measurements With OCT Angiography

The instrument used for OCT angiography (OCTA) images is based on the Optovue RTVue XR Avanti (Optovue, Inc., Freemont, CA, USA) to obtain split spectrum amplitude decorrelation angiography images. This instrument has an A-scan rate of 70,000 scans per second, using a light source centered on 840 nm and a bandwidth of 45 nm. Each OCTA volume contains 304 × 304 A-scans with two consecutive B-scans captured at each fixed position before proceeding to the next sampling location. The scan area was 3 × 3 mm. Each OCTA volume is acquired in 3 seconds and two orthogonal OCTA volumes were acquired so as to perform motion correction to minimize motion artifacts arising from microsaccades and fixation changes. Angiography information displayed is the average of the decorrelation values when viewed perpendicularly through the thickness being evaluated. If the image processed with motion correction software

Table 1. Summary Statistics for Axial Length, CFT, Equivalent Radius of the FAZ, MPOD, and Radius at Half Peak of MPOD

	Axial Length, mm	CFT, μm	Equivalent Radius, deg	Peak MPOD	Radius at Half Peak of MPOD, deg
Mean	24.5	206	1.015	0.578	1.11
Median	24.1	202	0.966	0.590	1.11
SD	1.5	19.2	0.221	0.128	0.31
Quartiles					
25th percentile	23.3	195	0.868	0.485	0.90
75th percentile	25.6	210	1.171	0.680	1.25

Mean, median, SD, and upper and lower quartiles for each parameter are provided.

demonstrated artifact in the form of doubling of vascular structures or sideways shearing of the image, then image acquisition was repeated.

Image analysis was performed using Adobe Photoshop cc (Adobe Systems, Inc., San Jose, CA, USA). The OCTA images from screen capture program integrated in the AngioView software (Optovue, Inc., Freemont, CA, USA) was used to obtain the vascular images. Gain and contrast were adjusted if necessary, using the brightness/contrast function, to allow clear delineation of the FAZ. The magic wand tool was then used to manually demarcate the boundaries of the FAZ. We used a tolerance of 15 and repeatedly used the magic wand tool to select the FAZ. If the selection spilled outside of the FAZ, the excess selection was trimmed using the lasso tool. If there were regions in the FAZ where pixel value exceeded the selected pixel value plus tolerance, then they were not selected by the magic wand tool. In these instances, the lasso tool was also used to manually select pixel outliers. The area measurement function was then used to determine the area of the FAZ in pixels^2 and this value was converted to μm^2 using scale conversion. This area was used to calculate a circle with an equivalent area and the equivalent radius (in degrees) of this circle as centered on the fovea was determined (Fig. 1). One degree was defined as 288 μm.[19] The equivalent radius of the FAZ was determined by two independent examiners and the average measurement was used for quantitative analysis.

Table 2. Bivariate Correlation Analyses Between the ER of the FAZ, Peak MPOD, Radius at Half Peak of MPOD, and CFT

		CFT	Equivalent Radius	Radius at Half Peak of MPOD
Equivalent radius	Pearson correlation	−0.601	—	—
	Significance, 2-tailed	<0.001	—	—
Radius at half peak of MPOD	Pearson correlation	−0.320	0.690	—
	Significance, 2-tailed	0.020	<0.001	—
Peak MPOD	Pearson correlation	0.345	−0.051	−0.126
	Significance, 2-tailed	0.011	0.702	0.343

Significant negative correlations were identified between CFT and equivalent radius of the FAZ and also CFT and the radius at half peak MPOD. Positive correlations were identified between equivalent radius of the FAZ and radius at half peak MPOD and also between CFT and peak MPOD.

Table 3. Interocular Correlations for Quantified Parameters

	Pearson Correlation	Significance, 2-Tailed
Equivalent radius of the FAZ	0.911	0.000
Peak macular pigment optical density	0.897	0.000
Radius at half peak of MPOD	0.920	0.000
CFT	0.806	0.000

There was significant intereye correlation for the size of the FAZ as measured using ER, peak MPOD, radius at half peak of MPOD, and CFT.

Statistical Analysis

Data were analyzed with descriptive statistics and to evaluate relationships between the size of the FAZ, CFT, peak MPOD, and radius at half peak of MPOD generalized estimating equations were used. A P value less than or equal to 0.05 was considered significant. The IBM SPSS version 21 statistical package was used (IBM Corporation, Armonk, NY, USA). Results are provided as mean ± SD.

Results

There were 59 eyes of 32 subjects, who were a mean of 31.3 ± 6.6 years; 15 were male. The mean axial length was 24.5 ± 1.5 mm and the mean CFT was 206.0 ± 19.2 μm. Signal score index on OCTA was greater than 60 out of 100 for all subjects (mean 80.1, SD 6.5).

The correlation between axial length and CFT was not significant ($P = 0.207$). The correlation between axial length and equivalent radius was also not significant ($P = 0.192$). The spatial relationships between FAZ topology, MPOD profile, and CFT for small and large FAZs are presented in Figures 2 and 3, respectively. The mean peak MPOD was 0.578 (SD 0.128) and the mean radius at half peak of MPOD was 1.11 degrees (SD 0.31; Table 1). Subpeaks in the MPOD spatial profile in a zone ranging from 0.5 to 1.0 degrees from the foveal center were more commonly observed in eyes with greater-sized FAZs (Fig. 3). Bivariate analysis showed significant negative correlation between CFT and both the equivalent radius of the FAZ and the radius at half peak of MPOD (Table 2). There was a high degree of bilateral symmetry for these parameters (Table 3).

Because many patients had bilateral imaging, the data were analyzed with generalized estimating equations. The results of these analyses are shown in Tables 4 to 6. The only significant predictor of the radius at half peak of MPOD was the size of the FAZ as expressed by the equivalent radius (Table 4). The only

Table 4. Analysis of Interrelationships with Generalized Estimating Equations Using Radius at Half Peak of MPOD as the Dependent Variable

			95% Wald Confidence Interval		Hypothesis Test	
Parameter	β	SE	Lower	Upper	Wald χ^2	P
Intercept	0.128	0.171	−0.208	0.464	0.559	0.460
Equivalent radius	0.968	0.177	0.621	1.316	29.850	<0.001

The only significant predictor was the size of the FAZ as expressed by the equivalent radius.

Table 5. Analysis of Interrelationships With Generalized Estimating Equations Using CFT as the Dependent Variable

			95% Wald Confidence Interval		Hypothesis Test	
Parameter	β	SE	Lower	Upper	Wald χ^2	P
Intercept	257.961	14.5947	229.356	286.566	312.407	<0.001
Equivalent radius	−51.445	12.679	−76.296	−26.595	16.463	<0.001

The only significant predictor was the size of the FAZ as expressed by the equivalent radius.

significant predictor of CFT was the equivalent radius of the FAZ (Table 5). The significant predictor for peak MPOD was CFT (Table 6).

Discussion

This study quantified the relationships between the topological characteristics of the FAZ and the MPOD spatial profile. The findings are as follows: (1) The equivalent radius of the FAZ is highly correlated with the radius at half peak of MPOD. (2) The equivalent radius of the FAZ is a significant predictor for CFT. (3) Central foveal thickness is a significant predictor for peak MPOD. (4) Interocular correlations for FAZ size, CFT, and the spatial profile of MPOD are significant. (5) Eyes with larger FAZs are more likely to have a secondary peak in their MPOD profile in a zone ranging from 0.5 to 1.0 degrees from the foveal center.

Theories concerning the function of macular pigments date back more than a century and the proposed roles for macular pigments include decreasing light scattering, reducing chromatic aberration, minimizing short wavelength–induced phototoxicity, increasing contrast, and protecting retinal structures against oxidative injury.[20–23] The absorption spectrum of macular pigments peaks at 460 nm,[2] which is in the blue region of the visible spectrum. Blue light is more likely to be scattered as per the Rayleigh equation and therefore it is plausible that macular pigments serve to reduce light scattering and consequently glare, but only for wavelengths that excite S-cones. Phototoxic injury is also more likely to occur with short wavelength light, and it is probable that macular pigments serve to protect against oxidative injury.[24,25]

Macular pigments are concentrated in the central fovea. Macular pigment density drops precipitously at the border of the vascularized portions of the retina, suggesting there may be a degree of functional overlap in the physiological role served by macular pigments and retinal vessels. Snodderly and Weinhaus[26] estimated that approximately 45% of the photons passing through the perifoveal retina of nonhuman primates would encounter one or more capillaries in their trajectory toward the photoreceptor layer. It is therefore likely that foveal capillaries and their contained red blood cells serve to reduce the risk of short-wavelength–induced phototoxicity and photo-oxidative damage. The proximity of capillary plexuses and macular pigments (within avascular retina) to the photoreceptor layer suggest that both structures serve to reduce photo-oxidative damage to outer segments that contain a high concentration of polyunsaturated fatty acids.[27]

Table 6. Analysis of Interrelationships With Generalized Estimating Equations Using Peak MPOD as the Dependent Variable

			95% Wald Confidence Interval		Hypothesis Test	
Parameter	β	SE	Lower	Upper	Wald χ^2	P
Intercept	0.107	0.1667	−0.219	0.434	0.414	0.52
CFT	0.002	0.0008	0.001	0.004	8.266	0.004

The only significant predictor was central foveal thickness (CFT).

The final stages of foveal development are characterized by the sequential centrifugal displacement of inner retinal layers following centripetal migration of cones toward the foveal center.[28] In this study, we found that CFT is inversely correlated to the equivalent radius of the FAZ, suggesting that the extent of antiparallel migration of inner retinal layers modulates the size of the FAZ. It is likely that differences in the spatial profile of MPOD relates to interindividual variations in neurovascular relationships that form during foveal development.[6] We showed that eyes with larger FAZs were more likely to have subpeaks in the MPOD spatial profile. Tariq et al.[18] showed that subpeaks and ring-like structures in the MPOD spatial profile are heritable. As subpeaks are related to the size of the FAZ, it is possible that genetic factors also determine the topological characteristics of the FAZ.

The functional advantage of MPOD subpeaks is unclear; however, they may relate to the degree of inner retinal layer separation and the extent to which the outer nuclear layer encroaches on the foveal center.[6] In a study involving nonhuman primates, Snodderly et al.[2] proposed that subpeaks in the spatial profile of MPOD correlate to regions in which macular pigment dominance shifts from the inner plexiform layer to Henle's fiber layer. The occurrence of subpeaks may have relevance for understanding pathophysiological mechanisms involved in retinal diseases, as the work by Dietzel et al.[29] showed that ring-like structures in the MPOD spatial profile were significantly more common in persons without age-related maculopathy. Meyer zu Westrup and colleagues[14] noted an inverse correlation between CFT and MPOD at 1 and 2 degree eccentricities. By extension, their findings are consistent with the present report. Because those eyes with a larger FAZ are more likely to have a subpeak in their MPOD profile and a lower CFT measurement, it would seem likely that MPOD at some eccentric location, such as 1 degree will be inversely related to CFT. Meyer zu Westrup et al.[14] did not find a correlation between CFT and MPOD at the 0 degree eccentricity and in this regard their results are different from our findings. The age of subjects in the study by Meyer zu Westrap et al.[14] was older than in our cohort (mean age 78.4 years). Furthermore, that study included participants with early AMD. Age[30] and retinal disease[31] are known to alter macular pigment distribution and are likely reasons why the results of Meyer zu Westrap et al.[14] were different from the findings in our present report. Liew et al.[32] found that CFT was positively correlated with

MPOD and their study examined healthy subjects between 17 and 50 years of age.

This study provides new information concerning the interrelationships between the FAZ, foveal metrics, and MPOD spatial profile and expands the findings of previous authors that have documented relationships between the FAZ and foveal anatomy.[9,14,33] The close interrelationship between the organization of foveal vessels and macular pigments is consistent with the hypothesis that macular pigments protect against photochemical damage. We, however, acknowledge several limitations of this study. The sample size in this study is restricted, and it will be important to evaluate a larger group of patients of different ages to determine if there is an age-dependent change in the relationship between FAZ size and the MPOD spatial profile. The relationships between sex, ethnicity, FAZ size, and MPOD spatial profile were also not examined this study. Expanding our understanding of the spatial profile of MPOD and its relationships to anatomical structures is likely to be important, as it has relevance for understanding pathogenic mechanisms important in aging and retinal diseases.

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References

1. Snodderly DM, Auran JD, Delori FC. The macular pigment. II. Spatial distribution in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:674–685.
2. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. I. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci*. 1984;25:660–673.
3. Nolan JM, Stringham JM, Beatty S, Snodderly DM. Spatial profile of macular pigment and its relationship to foveal architecture. *Invest Ophthalmol Vis Sci*. 2008;49:2134–2142.
4. Kirby ML, Galea M, Loane E, Stack J, Beatty S, Nolan JM. Foveal anatomic associations with the secondary peak and the slope of the macular pigment spatial profile. *Invest Ophthalmol Vis Sci*. 2009;50:1383–1391.
5. Hammond BR Jr, Wooten BR, Snodderly DM. Individual variations in the spatial profile of human macular pigment. *J Opt Soc Am A Opt Image Sci Vis*. 1997;14:1187–1196.
6. Tick S, Rossant F, Ghorbel I, et al. Foveal shape and structure in a normal population. *Invest Ophthalmol Vis Sci*. 2011;52:5105–5110.
7. Provis JM, Diaz CM, Dreher B. Ontogeny of the primate fovea: a central issue in retinal development. *Prog Neurobiol*. 1998;54:549–580.
8. Chui TY, Zhong Z, Song H, Burns SA. Foveal avascular zone and its relationship to foveal pit shape. *Optom Vis Sci*. 2012;89:602–610.
9. Chui TY, VanNasdale DA, Elsner AE, Burns SA. The association between the foveal avascular zone and retinal thickness. *Invest Ophthalmol Vis Sci*. 2014;55:6870–6877.
10. Nussbaum JJ, Pruett RC, Delori FC. Historic perspectives. Macular yellow pigment. The first 200 years. *Retina*. 1981;1:296–310.
11. Abadi RV, Cox MJ. The distribution of macular pigment in human albinos. *Invest Ophthalmol Vis Sci*. 1992;33:494–497.
12. Lima VC, Rosen RB, Prata TS, et al. Association of age and macular pigment optical density using dual-wavelength autofluorescence imaging. *Clin Ophthalmol*. 2013;7:685–690.
13. Creuzot-Garcher C, Koehrer P, Picot C, Aho S, Bron AM. Comparison of two methods to measure macular pigment optical density in healthy subjects. *Invest Ophthalmol Vis Sci*. 2014;55:2941–2946.
14. Meyer zu Westrup V, Dietzel M, Pauleikhoff D, Hense HW. The association of retinal structure and macular pigment distribution. *Invest Ophthalmol Vis Sci*. 2014;55:1169–1175.
15. Trieschmann M, Spital G, Lommatzsch A, et al. Macular pigment: quantitative analysis on autofluorescence images. *Graefes Arch Clin Exp Ophthalmol*. 2003;241:1006–1012.
16. Trieschmann M, Heimes B, Hense HW, Pauleikhoff D. Macular pigment optical density measurement in autofluorescence imaging: comparison of one- and two-wavelength methods. *Graefes Arch Clin Exp Ophthalmol*. 2006;244:1565–1574.
17. Berendschot TT, van Norren D. Macular pigment shows ringlike structures. *Invest Ophthalmol Vis Sci*. 2006;47:709–714.
18. Tariq A, Mahroo OA, Williams KM, et al. The heritability of the ring-like distribution of macular pigment assessed in a twin study. *Invest Ophthalmol Vis Sci*. 2014;55:2214–2219.
19. Drasdo N, Fowler CW. Non-linear projection of the retinal image in a wide-angle schematic eye. *Br J Ophthalmol*. 1974;58:709–714.
20. Schultze M. *Ueber den gelben Fleck der Retina, seinen Einflussauf normales Sehen und auf auf Farbenblindheit [On the Yellow Spot of the Retina: Its Influence on Normal Vision and on Colour Blindness]*. Bonn: Verlag von Max Cohen & Sohn; 1866.
21. Schultze M. The retina. In: Stricker S, ed. *Manual of Human and Comparative Histology*. London: New Syndenham Society; 1873.
22. Khachik F, Bernstein PS, Garland DL. Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci*. 1997;38:1802–1811.
23. Bernstein PS, Khachik F, Carvalho LS, Muir GJ, Zhao DY, Katz NB. Identification and quantitation of carotenoids and their metabolites in the tissues of the human eye. *Exp Eye Res*. 2001;72:215–223.
24. Hammond BR, Johnson BA, George ER. Oxidative photodegradation of ocular tissues: beneficial effects of filtering and exogenous antioxidants. *Exp Eye Res*. 2014;129:135–150.
25. Snodderly DM. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am J Clin Nutr*. 1995;62:1448S–1461S.
26. Snodderly DM, Weinhaus RS. Retinal vasculature of the fovea of the squirrel monkey, *Saimiri sciureus*: three-dimensional architecture, visual screening, and relationships to the neuronal layers. *J Comp Neurol*. 1990;297:145–163.
27. Anderson RE, Benolken RM, Dudley PA, Landis DJ, Wheeler TG. Proceedings: Polyunsaturated fatty acids of photoreceptor membranes. *Exp Eye Res*. 1974;18:205–213.
28. Springer AD, Hendrickson AE. Development of the primate area of high acuity, 3: temporal relationships between pit formation, retinal elongation and cone packing. *Vis Neurosci*. 2005;22:171–185.
29. Dietzel M, Zeimer M, Heimes B, Pauleikhoff D, Hense HW. The ringlike structure of macular pigment in age-related maculopathy: results from the Muenster Aging and Retina Study (MARS). *Invest Ophthalmol Vis Sci*. 2011;52:8016–8024.

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30. Baptista AM, Nascimento SM. Changes in spatial extent and peak double optical density of human macular pigment with age. *J Opt Soc Am A Opt Image Sci Vis*. 2014;31:A87–A92.
31. Kaya S, Weigert G, Pemp B, et al. Comparison of macular pigment in patients with age-related macular degeneration and healthy control subjects—a study using spectral fundus reflectance. *Acta Ophthalmol*. 2012;90:e399–e403.
32. Liew SH, Gilbert CE, Spector TD, et al. Central retinal thickness is positively correlated with macular pigment optical density. *Exp Eye Res*. 2006;82:915–920.
33. Dubis AM, Hansen BR, Cooper RF, Beringer J, Dubra A, Carroll J. Relationship between the foveal avascular zone and foveal pit morphology. *Invest Ophthalmol Vis Sci*. 2012;53:1628–1636.

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