Macular Pigment Parameters in Patients with Macular Telangiectasia (MacTel) and Normal Subjects: Implications of a Novel Analysis

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Purpose. To evaluate the spatial distribution and total amount of macular pigment (MP) in patients with idiopathic macular telangiectasia type 2 (MacTel) compared to healthy subjects.

Methods. Totals of 53 MacTel patients and 38 normal subjects underwent macular pigment optical density (MPOD) measurement using a 2-wavelength autofluorescence (2-AF) technique. The peak MPOD and total MP (sum of pixel OD values) were measured within the central 21 degrees. Data were correlated with motion photometry in a cohort of normal subjects.

Results. A Bland-Altman analysis revealed minimal differences between psychophysical and 2-AF measurements of MPOD (bias = 0.025, SD = 0.06, N = 156 values). In the normal comparison group, 2-AF MPOD peak had a median value of 0.57 (range 0.21–0.93), and median eccentricity of the peak was 0.19 degrees (range 0.00–0.41). In the MacTel group, MPOD peak had a median value of 0.08 (range 0.01–0.26), and median eccentricity of the peak was 5.04 degrees (range 0.18–7.27). The median total amount of MP within the central 21 degrees was greater for normal subjects (4802, range 2362–9215) than for the patients (2938, range 142–7198), but there was marked overlap between the groups. Comparison of the total amount within the central 8, 12, or 16 degrees to that within the central 21 degrees revealed underestimation of up to 68% (median 53%), 42% (27%), and 24% (8%), respectively.

Conclusions. Most MacTel patients have a normal total complement of MP with an abnormal paracentral distribution. The study highlights the limitations of MP measurement techniques that assume minimal MP at eccentricities less than 10.5 degrees. (*Invest Ophthalmol Vis Sci.* 2012;53:6568–6575) DOI:10.1167/iovs.12-9756

Idiopathic macular telangiectasia type 2a (MacTel) was first well characterized by Gass et al. in 1982[1] and a modified classification was published in 1993[2] based on clinical examination and fluorescein angiography. Histopathology confirmed the presence of retinal vascular changes.[3] More recently, the MacTel research collaboration (details available online at http://www.mactelresearch.org/) applied new imaging techniques to large numbers of patients and characterized further the disorder according to optical coherence tomography (OCT) changes,[4,5] alterations in cone density on imaging with adaptive optics,[6] and alterations in confocal blue light reflectance imaging.[7] New insights have been gained into the natural history[8] and histopathology[9] of the disorder, and a possible inherited cause is under investigation with several candidate genes having been excluded.[10] These studies have contributed to a change in the understanding of the underlying disease mechanisms of MacTel from that of a predominantly retinal vascular disease to a disease with a significant neurodegenerative component. Although anti-VEGF treatment has been proven effective in normalization of vascular alterations in MacTel, progressive photoreceptor disruption and worsening of function have been documented.[11]

A unique, abnormal parafoveal distribution of macular "luteal" pigment (MP) has been described in MacTel.[7,9,12,13] The central macular hyperfluorescence that is visible in fundus autofluorescence images as the disease develops has been ascribed to the abnormal paracentral distribution of macular pigment,[14] and may correlate with functional impairment and disease severity.[15] Recent studies have shown parafoveal macular pigment optical density (MPOD) levels that are comparable with those in some healthy subjects at corresponding eccentric retinal locations,[13] and have raised the possibility that the largely annular distribution results from localized loss of the central MP component. However, few individuals have been examined, not all healthy subjects have a significant lateral (eccentric) MP component,[16] and the underlying cause of abnormal MP distribution in MacTel remains unknown.

The aim of our study was to compare the spatial distribution and total complement of MP in MacTel patients to those in a group of normal subjects using a novel analysis of 2-wavelength fundus autofluorescence (2-AF) images, to investigate in more detail the abnormal MP distribution. Data from the normal subjects were used additionally to test the limitations of more traditional methods of MP assessment by simulating the effects of lateral pigmentation on computations of relative MPOD.

Methods

This research adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee. Informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study.

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As a part of the Macular Telangiectasia Project (MacTel project, details available online at www.mactelresearch.org) a cohort of patients and volunteers were assessed at Moorfields Eye Hospital, London, UK, and underwent MPOD imaging studies. Data were analyzed retrospectively. We examined 53 eyes of 53 patients with MacTel and 38 eyes of 38 healthy subjects. Only right eyes were assessed. The mean age of the MacTel subjects was 61.1 ± 9.4 years (range 36–75) and the mean age of control subjects was 46.5 ± 13.9 years (range 18–76). The patients were graded following the classification described by Gass et al.[1,2]

All comparison subjects and patients underwent 2-AF imaging using a modified confocal scanning laser ophthalmoscope (Heidelberg HRA, Heidelberg, Germany), according to previously described methods.[12,13,17–20] In brief, images were obtained using two argon lasers (radiation 488 and 514 nm) and aligned automatically according to anatomic landmarks, such as the retinal blood vessels. A map of the relative MPOD of each pixel was obtained by digital subtraction of the log autofluorescence data at the two wavelengths. The MPOD maps were used to compute mean 2-dimensional distribution profiles in all patients and normals relative to the mean value for an annulus at 10.5 degrees eccentricity. The sum of pixel OD values within the whole of the 10.5-degree area was used to measure the total amount (equivalent "volume") of MP in different individuals. The spatial profiles of MP obtained from normal subjects were used additionally to model the effects of laterally distributed macular pigmentation on computations of MPOD relative to reference locations at 4, 6, and 8 degrees eccentricity. These eccentricities (4–8 degrees) cover the range of reference locations used in most psychophysical and imaging studies of MP (see Discussion).

For validation purposes, measurements of MPOD made in 12 healthy subjects using the 2-AF imaging technique were compared to those made using the established psychophysical technique of minimum motion photometry.[16,21–23] The minimum motion measurements of MPOD were made using 13 stimulus fields at different retinal locations. Square wave gratings (components 460 and 580 nm, spatial frequency 0.38 cycles/degree) moved horizontally across 2 central circular fields (diameter 0.9 and 2.2 degrees) and across 11 annular segments at eccentricities 0.8–7.5 degrees above the fovea. The 450 nm light was added to both elements to saturate S-cones. Grating velocity was held constant at 37 degrees/sec. The radiance of the 580 nm component was adjusted to minimize perceived motion. Relative OD was calculated as log (Rref/R), where Rref is the mean radiance of the 580 nm comparison stimulus for the most eccentric location (7.5 degrees) and R is the radiance setting at any location. The 2-AF images were used to quantify MPOD over annuli of equal width and eccentricity to areas tested psychophysically, relative to a reference eccentricity of 7.5 degrees, allowing direct comparison with the psychophysical technique.

Results

Comparison of MPOD Measurement Methods

There was high correlation and close correspondence between psychophysical and 2-AF measurements of MPOD (Fig. 1a, slope = 1.00, $r = 0.94$, $P < 0.005$, $N = 12$ eyes of 12 subjects, 156 values). A Bland-Altman plot of the difference between the two estimates against the average MPOD revealed minimal differences (Fig. 1b, bias = 0.025, SD = 0.06).

Consistency of 2-AF Measurements

Five representative normal subjects underwent 2-AF testing on 3 occasions and the images were used to assess intra-subject measurement variability. Peak MPOD differed from the mean value by a mean of 6.7% (range 4.0–13.1%, median 5.3%). In terms of the total amount of MP, the mean difference was 9.1% (range 1.7–17.3%, median 7.6%). Comparison of SD at 0.5, 5, and 9 degrees revealed a mean relative SD (SD/mean) of 19.8%, 20.7%, and 17.0%, respectively.

Comparison of Normal and Patient MP Parameters

Figure 2 shows 2-AF OD maps and corresponding spatial profiles in 4 normal subjects and in 4 MacTel patients. In the control group 2-AF assessment revealed an MPOD peak with a median value of 0.57 and a range of 0.21–0.93 (Fig. 3a). The median eccentricity of the peak was 0.19 with a range of 0.00–0.41 degrees (Fig. 3b). The shape of the MP spatial distribution varied; lateral MPOD dropped to 0.05 at a median eccentricity of 6.3 degrees (range 3.05–8.03 degrees, Fig. 3c) and to 0.02 at a median eccentricity of 7.8 degrees (range 5.57–9.38 degrees). Three normal subjects had a prominent double peak in the profile at 0.41–0.94 degrees eccentricity (Fig. 2d).

In the MacTel patients, foveal MP was mostly undetectable (Figs. 2, 3b). There was a parafoveal (eccentric) MP distribution in 50 of 53 cases, with 22 of these patients showing a clear nasal predominance. In one case the nasal MP extended to the fovea (Fig. 2h). Mean 2-dimensional spatial profiles revealed a broad MP peak with a maximum MPOD of 0.01–0.23 (median 0.07, Fig. 3a) at eccentricities of between 1.41 and 7.27 (median 5.18) degrees eccentricity (Fig. 3b). In patients with an eccentric peak MPOD greater than 0.05 the lateral MPOD value dropped to 0.05 at a median eccentricity of 6.80 degrees (range 2.46–8.03 degrees, Fig. 3c, $N = 43$) and to 0.02 at a median eccentricity of 8.32 degrees (range 5.92–9.49 degrees, $N = 53$).

A minority of MacTel cases (3 patients) had an MP distribution indistinguishable from that seen in the normal subjects with a peak at the fovea (MPOD 0.11–0.26). Peak foveal MPOD was within 2 ($N = 2$) or 2.5 SDs of the normal mean. In these cases the lateral MP component dropped to an OD value of 0.05 at a median eccentricity of 6.55 degrees (range 1.82–7.15 degrees) and to 0.02 at 8.32 degrees.

There was a statistically significant difference between the normal ($N = 38$) and patient ($N = 53$) groups in terms of mean peak MPOD and mean eccentricity of the peak MPOD according to a two-sample Wilcoxon rank-sum (Mann-Whitney) test ($P < 0.0001$). There was strong evidence of a difference between the two groups in terms of MPOD at 5 degrees eccentricity (t-test, $P = 0.0391$).

The mean total amount of MP within the central 21 degrees was greater for normal subjects (median 4802, range 2362–9215) than for patients with an annular MP distribution with (median 3519, range 513–7198) or without (median 2261, range 142–4480) a predominant nasal component, but there was marked overlap between normal and patient groups (Fig. 3d). The peak MPOD values correlated strongly with the total complement of MP in the patients without foveal MP ($r^2 = 0.82$, $P < 0.01$, $N = 49$). In the control group the corresponding correlation was weaker ($r^2 = 0.42$, $P < 0.01$) and peak MPOD could not be used to predict reliably the total complement (Fig. 4).

Comparison of Disease Severity with MP Parameters

Disease severity was classified as grade 1 (4 eyes), grade 2 (11 eyes), grade 3 (17 eyes), grade 4 (17 eyes), or grade 5 (4 eyes). Seven patients had mildly asymmetric disease (differing by one grade) and two had more marked asymmetry (differing by two grades).

Neither peak MPOD nor the lateral extent of MP correlated significantly with disease severity. Cross-sectional analysis showed that the median total complement of MP was reduced

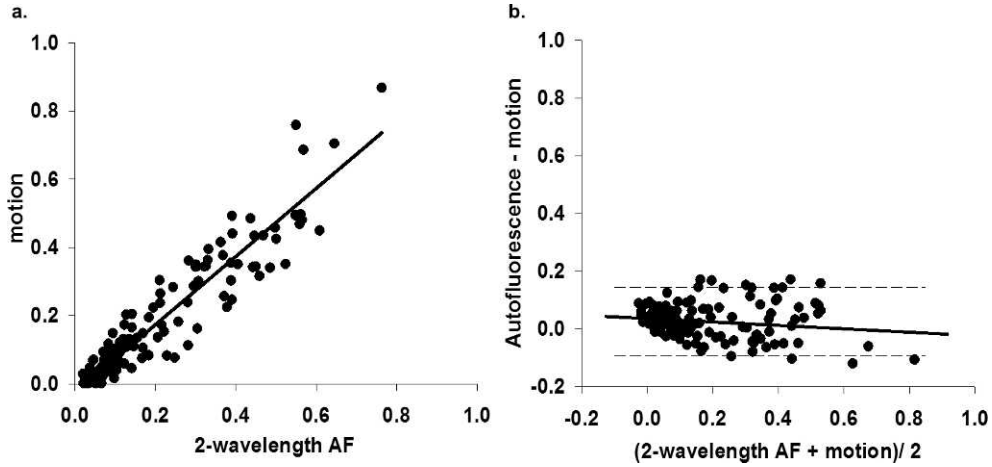

Figure 1. (**a**) Comparison of relative MPOD values obtained using minimum motion photometry with those estimated from profiles derived from 2-wavelength AF images. (**b**) Corresponding Bland Altman plot. $N = 12$ subjects. *Broken lines*: show 2 SDs either side of the mean difference.

progressively from stages 2 to 5 but there was wide variability and no significant correlation (Fig. 5); the two patients with stage 1 disease included an individual with very low levels of total MP. Disease severity was stages 1, 2, and 4 in the three MacTel patients with a peak MPOD at the fovea.

Simulation Using a Less Eccentric Reference Location

Figure 6a models the effects of lateral pigmentation on computations of relative MPOD in healthy subjects. Reducing the eccentricity of the reference location to 4, 6, or 8 degrees leads to underestimation of peak optical density by up to 27% (median 18%), 16% (median 10%), and 9% (median 4%), respectively; underestimation tended to be greater for subjects with low peak levels of MPOD (Fig. 6a). The effects of using a less eccentric reference had a proportionately greater effect on computations of total MP; comparison of the total complement within the central 10.5 degrees with the amount in the central 4, 6, or 8 degrees led to underestimation of up to 68% (median 53%), 42% (median 27%), and 24% (median 8%), respectively (Fig. 6b).

Discussion

Our study compares multiple MP parameters in a large group of MacTel patients to those in healthy subjects, including a measurement of the total MP complement. To our knowledge measurement of total MP has not been performed in previous studies. The study additionally expands our knowledge by describing MP parameters relative to a more eccentric retinal location than is usual, highlighting the limitations of more typical MP measurement methods.

MPOD traditionally has been measured using subjective techniques, such as minimum flicker photometry, color matching, and minimum motion photometry. A potential problem with these techniques is that psychophysical testing can be difficult for some observers and for patients with visual impairment or macular disease, particularly if multiple measurements are required to obtain a spatial profile, to assess

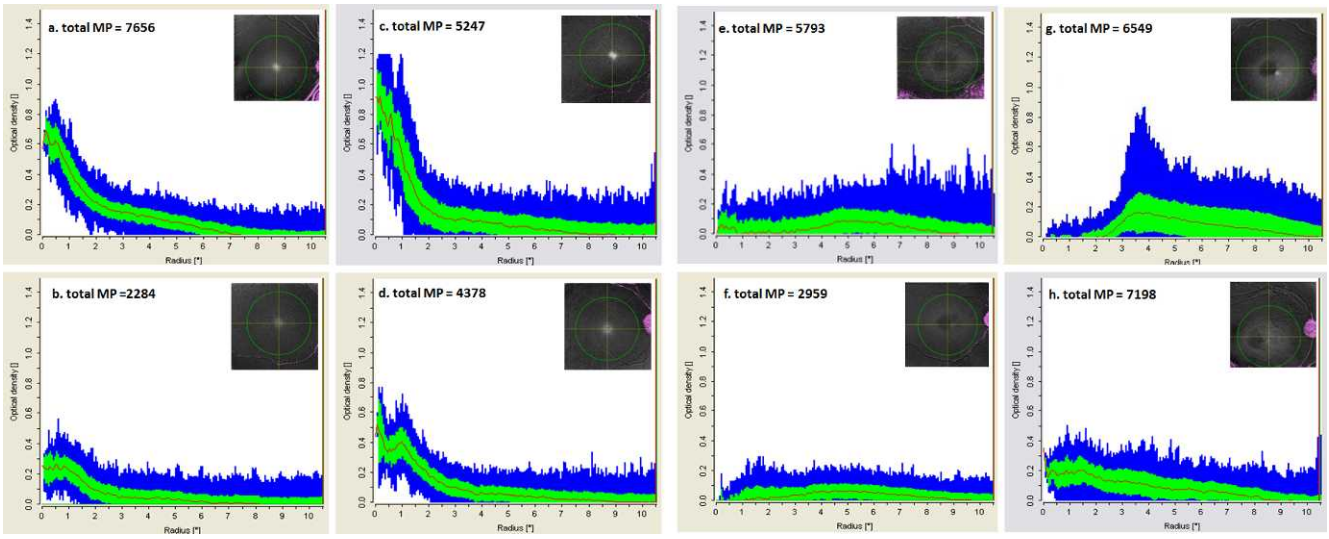

Figure 2. Comparison of MPOD profiles obtained using 2-wavelength fundus autofluorescence in four representative healthy subjects (**a**–**d**) with those in patients with MacTel type 2 with a predominantly annular (**e**–**g**) or more complex MP distribution with both annular and large nasal components (**h**). In one patient there was residual MP at the fovea (**e**) and in one case nasal MP extended to the fovea (**h**). The corresponding 2-wavelength image (*inset*) and measured total amount of MP is shown in each case.

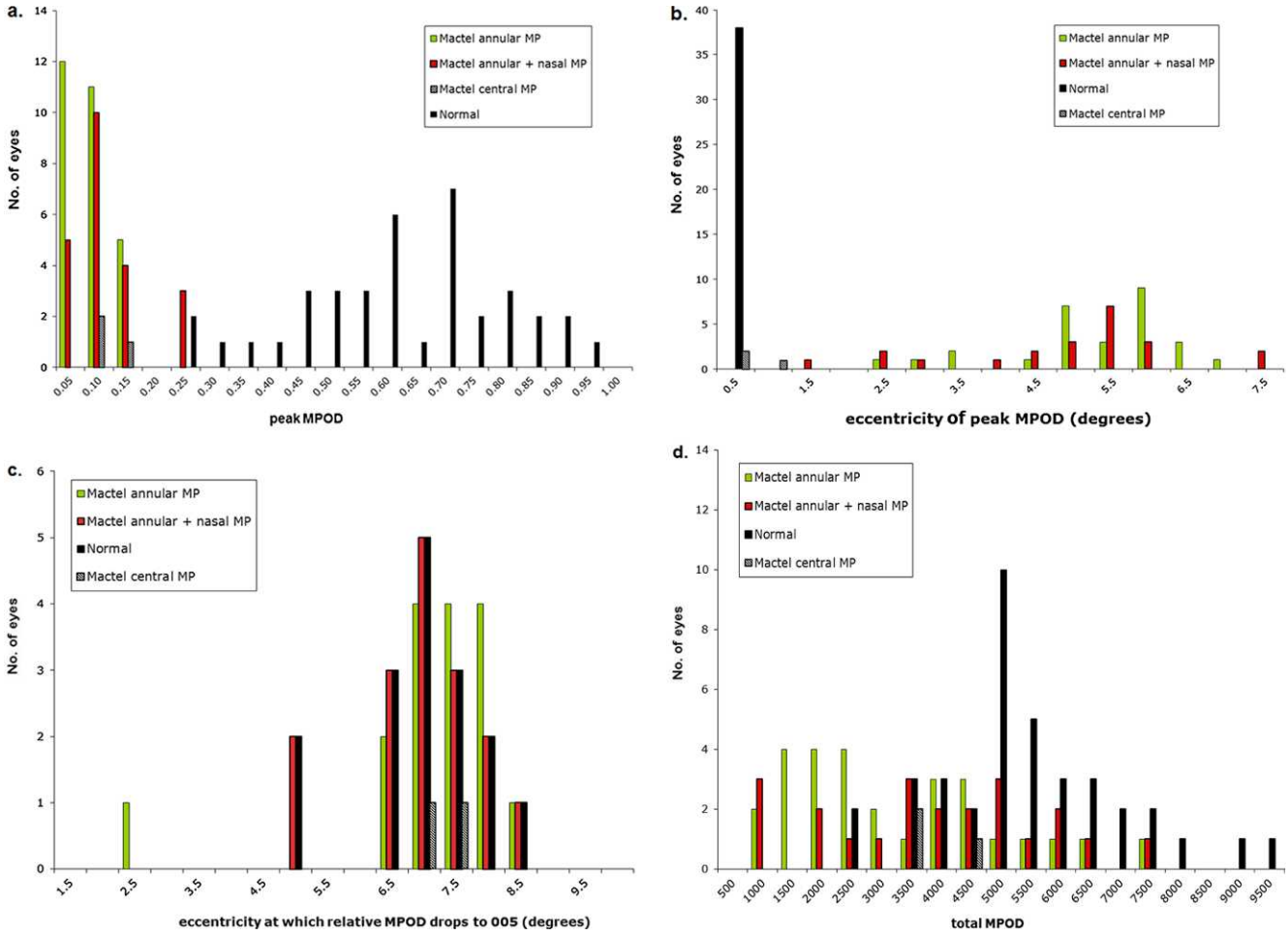

Figure 3. Histograms showing the relative peak MPOD (**a**), eccentricity of peak MPOD (**b**), eccentricity at which the lateral MPOD falls below 0.05 (**c**), and measured total MP complement (**d**) in the normal subjects (38 eyes) and MacTel patients (53 eyes). Peak MPOD was 0.05 or less in 11 MacTel patients excluded from Figure 3c.

radial symmetry, or to obtain an estimate of total MP. New fundus imaging and objective measurement techniques offer advantages and some disadvantages,[18,24] but must be validated against established methods. In our study, comparison of the 2-wavelength technique with minimum motion photometry revealed good correspondence and closer agreement than did recent comparisons of 2-AF with heterochromatic flicker photometry.[25] This may relate partly to the comparison technique; motion photometry is based on the phenomenon of motion nulling observed in the absence of luminance contrast,[26–28] and it is noted that direct comparison between minimum-flicker and minimum motion matches made by naive subjects indicates that errors for motion are half those for flicker.[29]

With a few exceptions, early studies of MP in normal subjects measured a peak foveal MPOD and ignored other characteristics, including the lateral extent, shape of the spatial profile, radial symmetry, and total amount of MP (analogous to volume). In healthy subjects the total measured amount cannot be predicted reliably from the peak value (Fig. 4) and this corroborates the findings of a previous psychophysical study that estimated total MP by numerical integration of 2-dimensional MP profiles.[16] There is evidence that paracentral MP may be elevated by dietary modification,[30–32] but the lateral spatial profile and the total MP complement continue to be neglected in recent supplementation[33–37] and phenotype-genotype[38,39] studies. The data from our study highlight the importance of measuring lateral pigmentation accurately, as small changes in eccentric MPOD may have a marked impact on the total complement of MP in health and disease, consistent with the high correlation between the peak of eccentric MPOD and corresponding values of total MP in the MacTel patients (Fig. 4). A predictable or linear decline in MP with eccentricity should not be assumed and our study warns against the measurement of "spatial profiles" using few eccentric measurements or methods that only estimate MP over locations that approach that of the reference. Similarly, "area under the curve" estimates of MP could give a grossly misleading estimate of the total MP complement.

Our study confirmed that most MacTel patients have an abnormal distribution of MP without a central peak and with a significant parafoveal MP component. Averaged 2-dimensional spatial profiles provided a convenient method of quantifying MP although analysis of the 2-AF MP image is required to assess radial MP asymmetry, either visually or by deriving multiple profiles.[12,20,40] The parafoveal MP had a nasal predominance in many MacTel type 2 patients, consistent with previous reports.[13,40] The MP can extend more laterally than in many healthy subjects, and constitutes a total MP endowment that is more variable than normal and that shows marked overlap with control values. The possible explanations for these observations would include abnormal eccentric accumulation of MP or disruption of the retinal mechanisms that normally mediate or maintain MP deposition. Previous studies showed

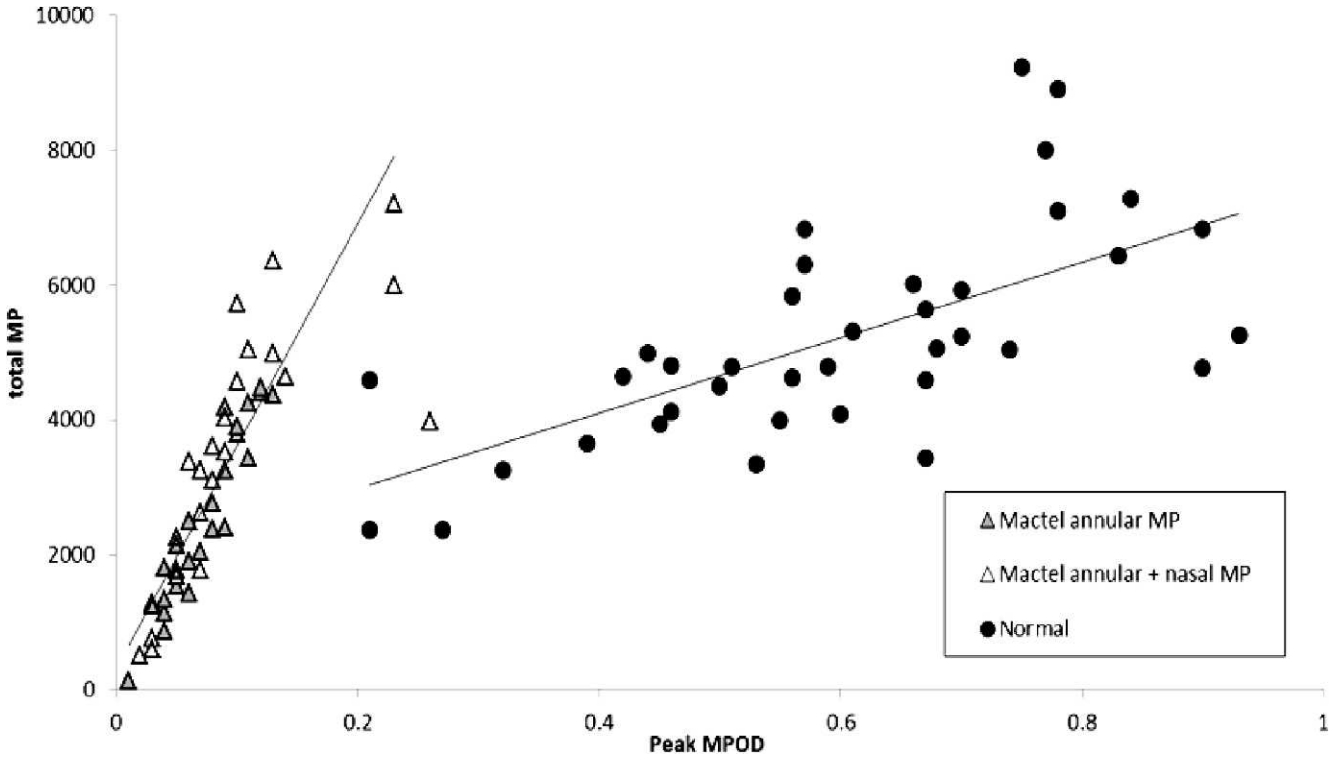

Figure 4. Comparison of peak MPOD with the total MP in normal subjects (*filled circles*) and MacTel patients with an annular MP distribution (*open triangles*) or annular + nasal MP distribution (*filled triangles*). Linear regression lines are for normal subjects and for the combined group of MacTel patients. See text for details.

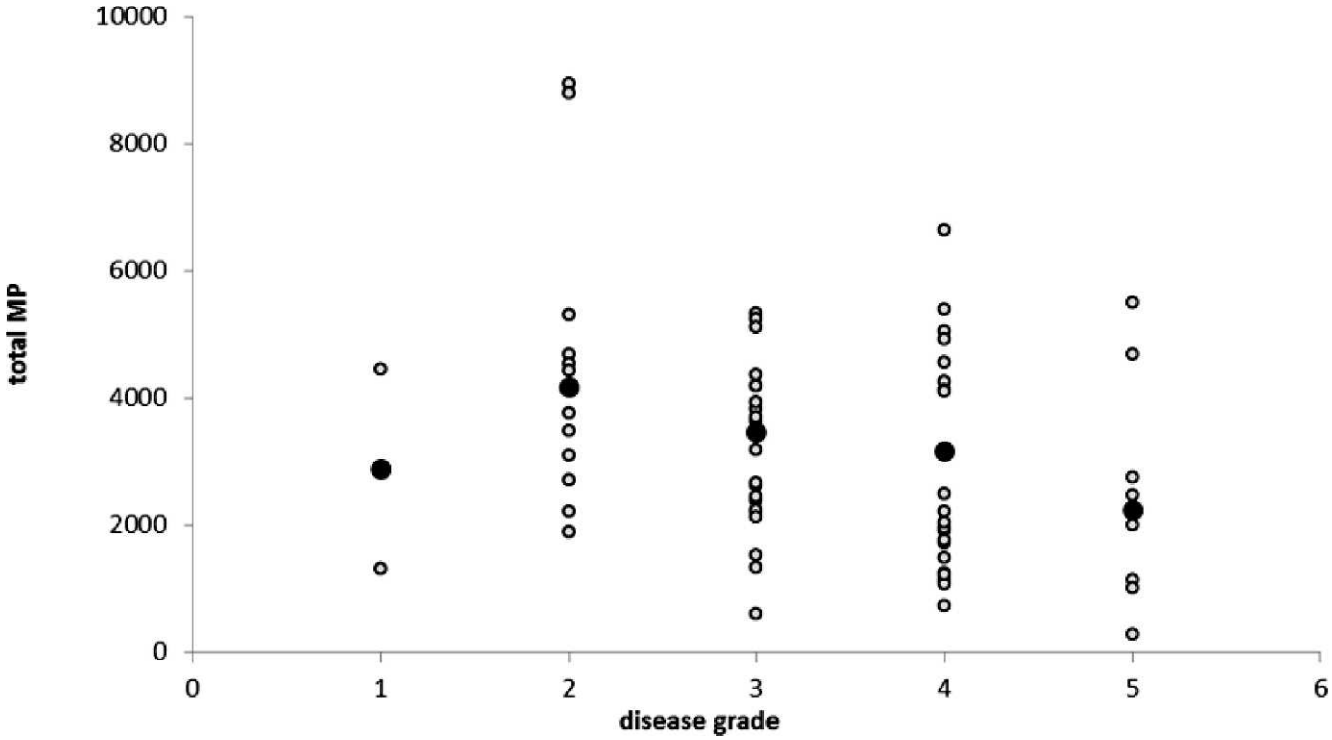

Figure 5. Comparison of disease grade (Gass classification) with the measured total complement of MP. Data points show values for each patient (*grey circles*) and median values for each stage of disease (*filled circles*).

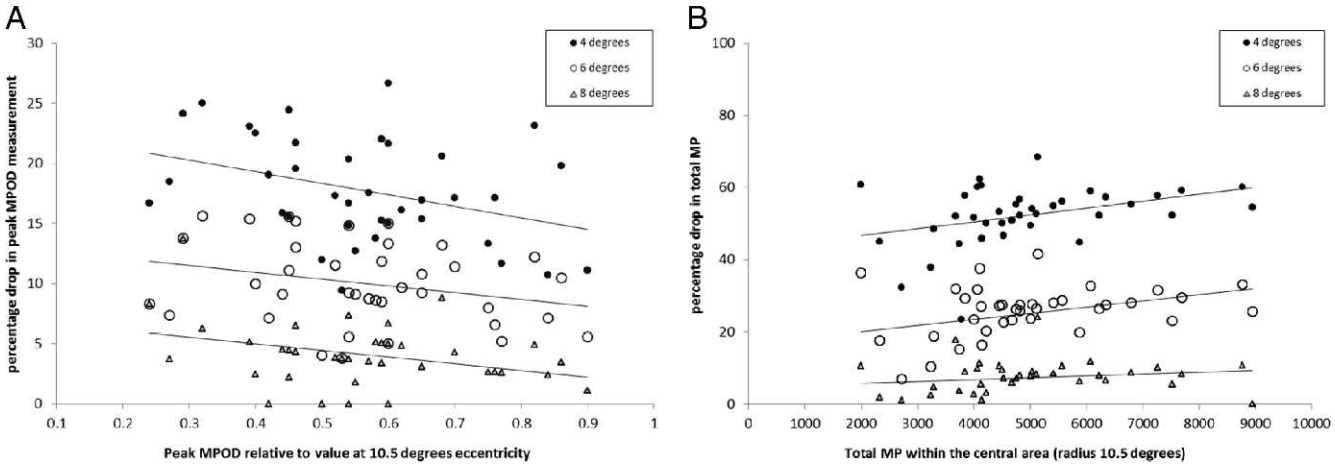

Figure 6. (**a**) The percentage drop in peak foveal MPOD values when computed relative to reference locations at 4, 6, or 8 degrees eccentricity compared to 10.5 degrees eccentricity. (**b**) The percentage drop in peak total MPOD when measured within the central 8, 12, or 16 degrees compared to the total amount measured within the central 21 degrees.

that the parafoveal MP in MacTel could resemble that seen in healthy subjects at corresponding eccentric retinal locations,[12,13] and it was suggested that this may represent loss of foveal MP from the diseased retina.[12] Our study confirmed that not all healthy subjects have a significant MP component extending laterally (Fig. 3c) and showed that the amount of parafoveal MP in MacTel patients may exceed the total complement in healthy subjects (Fig. 3d). It is noted that total MP in some MacTel patients may be underestimated if trace levels at 10.5 degrees exceed those at less eccentric locations, and there was a suggestion of this in some patients (Fig. 2g). The pathophysiology of abnormal pigmentation in MacTel type 2 is not known, but the normal shape of the MP distribution in MacTel type 1 suggests that the typical retinal vascular (telangiotic) changes are not directly responsible.[9,12] Müller cell loss in the Henle fiber layer occurs in central areas lacking MP[9] and a role of Müller cells in MP sequestration has been postulated.[41] The lack of correlation between MP parameters and disease severity suggests that changes in MP occur early in the disease process.[42]

Computations of relative MPOD usually require a reference setting at a location outside the pigmented area, and in psychophysical assessments this location must be central enough to minimize the confounding influence of Troxler's fading and rod intrusion. Compromise locations at 4–8 degrees eccentricity have been used routinely in psychophysical and imaging studies[15,16,18,20–22,30,31,33–38,43–50] with few exceptions,[51] although MPOD may be underestimated significantly in healthy subjects with significant lateral MP (Fig. 6a) and can result in negative OD values in MacTel patients with significant paracentral pigment.[13] Being a relative measure, negative OD values only occur when MP is depleted centrally. Additionally, MacTel patients often have less MP temporally and psychophysical characterization of MP distribution would require multiple spatial profiles. Fundus imaging methods offer the possibility of computing MPOD relative to a more eccentric and less-pigmented reference location, beyond areas amenable to psychophysical measurement, and it is perhaps surprising that so few imaging studies have exploited this advantage. Our study used a reference location at 10.5 degrees eccentricity, which is more eccentric than in most similar studies of MP.

It is tempting to speculate that eccentric MP in MacTel is due to abnormal paracentral uptake, abnormal binding, or centrifugal displacement of central pigment rather than being a lateral remnant of a previously normal MP distribution centered on the fovea, that is a deficiency of pigment. Until the role of MP in the etiology of MacTel is understood, we plan to continue to assess pigmentation in terms of peak density, total MP, and distribution. The current data confirmed that 2-wavelength fundus autofluorescence is a valid and practical method of measuring MPOD and distribution, and provides a measure of total MP without assuming radial symmetry; variables that cannot always be quantified psychophysically. The study additionally highlights the limitations of imaging and most psychophysical studies that have computed MPOD relative to less eccentric reference locations.

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