Abnormal retinal reflectivity to short-wavelength light in type 2 idiopathic macular telangiectasia.

[1A, 2*]Ferenc B. Sallo, MD, PhD; [1A]Irene Leung, BA; [3]Meike Zeimer, MD; [4]Traci E. Clemons, PhD; [1A, 2]Adam M. Dubis, PhD; [2]Marcus Fruttiger, PhD; [3]Daniel Pauleikhoff, MD, PhD; [5]Emily Y. Chew, MD; [1A, 1C]Catherine Egan, MD; [6, 7]Tunde Peto, MD, PhD; [1B]Alan C. Bird, MD and the MacTel Study group.

[1]Moorfields Eye Hospital, London, United Kingdom,
[A]Department of Research and Development, [B]Inherited Eye Disease, [C]Medical Retina Service
[2]UCL Institute of Ophthalmology, London, United Kingdom
[3]St. Franziskus Hospital, Münster, Germany
[4]The EMMES Corporation, Rockville, Maryland, USA
[5]National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA
[6]NIHR Biomedical Research Center for Ophthalmology, at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, UK
[7]Queen's University Belfast Faculty of Medicine Health and Life Sciences, Belfast, UK

[*] Correspondence should be directed to:

Ferenc B Sallo MD, PhD
The Reading Center
Department of Research and Development
Moorfields Eye Hospital NHS Foundation Trust
162 City Road, London, EC1V 2PD, UK
phone: +44 207 566 2815
Fax: +44 207 608 6925
email: Ferenc.Sallo@moorfields.nhs.uk

Financial support: The authors wish to thank the Lowy Medical Research Institute (LMRI) for providing support for funding this study. The sponsor had no role in the design or conduct of this research but did participate in the approval of the manuscript.

Proprietary interest: no conflicting relationship exists for any author.

Running head: Short-wavelength reflectance imaging in MacTel.

Acknowledgements: Many thanks are due Mr Vincent Rocco for valuable insights into the technical aspects of multimodal imaging with special respect to BLR imaging.

Key Words
blue light reflectance, angiography, degeneration, imaging, MacTel, macular pigment, Müller cells, OCT, retina, telangiectasia.

Summary statement
MacTel affects a well-defined region of the macula, indicating localized susceptibility. Our findings indicate that abnormal metabolic handling of luteal pigment and physical changes giving rise to increased reflectance are widespread in the macula throughout the natural history of the disease, precede other changes and are relevant to early diagnosis.

ABSTRACT

Purpose:
Macular Telangiectasia Type 2 (MacTel) is a bilateral, progressive, potentially blinding retinal disease characterized by vascular and neurodegenerative signs, including an increased parafoveal reflectivity to blue light (BLR). Our aim was to investigate the relationship of this sign with other signs of MacTel in multiple imaging modalities.

Methods:
Participants were selected from the MacTel Study, based on a confirmed diagnosis and the availability of images. The extent of signs in BLR, fluorescein angiographic, optical coherence tomographic (OCT) and single- and dual-wavelength autofluorescence (DWAF) images were analyzed.

Results:
A well-defined abnormality of the perifovea is demonstrated by dual wavelength autofluorescence and blue light reflectance in early disease. The agreement in area size of the abnormalities in DWAF and in BLR images was excellent, for right eyes: ρ=0.917 ($p<0.0001$, 95%CI 0.855-0.954, n=46) and for left eyes: ρ=0.952 ($p<0.0001$, 95%CI 0.916-0.973, n=49). Other changes are less extensive initially and expand later to occupy that area and do not extend beyond it.

Conclusion:
Our findings indicate that abnormal metabolic handling of luteal pigment and physical changes giving rise to increased reflectance are widespread in the macula throughout the natural history of the disease, precede other changes and are relevant to early diagnosis.

INTRODUCTION

Macular Telangiectasia Type 2 (MacTel) is a bilateral, slowly progressive retinal disease of unknown pathogenesis, potentially leading to a loss of central vision.[1] Previous clinical and histopathological data suggest a hereditary cause and a central role for retinal Müller cells in the pathogenesis of the disease.[2-4]

The phenotype of MacTel is limited to the perifoveal region and is characterized by both vascular and neurodegenerative changes. Color and red-free fundus photographic signs include a loss of retinal transparency, retinal crystals at the level of the internal limiting membrane (ILM) and in later disease pigment plaques in the mid-retina. Fundus fluorescein angiographic (FFA) signs include dilated, blunted and right-angle veins, dilated deep retinal capillaries and hyperfluorescence (early leakage, late staining) but without an increase in retinal thickness. In late disease subretinal neovascularization may occur.[5-7]

More recent imaging modalities have provided valuable insight into previously little or unknown characteristics of the phenotype (Figure 1). A loss of retinal transparency is considered an early sign in MacTel (driving stage 2 in the severity classification of the disease devised by Gass and Blodi[8]), indeed it may be the first detectable manifestation of the disease. Reflective imaging using short wavelength light ('blue light reflectance imaging', BLR) provides a superior tool for detecting and recording this phenomenon.[9-11]

Short-wavelength (488nm, blue) autofluorescence imaging may demonstrate increased signal in the parafoveal region in early disease, and later decreased autofluorescence corresponding to intraretinal perivascular pigment clumps or plaques.[5] Using dual (488+512nm) wavelength autofluorescence (DWAF) imaging,[12-14] a redistribution of macular (luteal) pigment (MP) in a specific pattern can be demonstrated. This pattern typically starts with a wedge-shaped depletion of MP just temporal of the foveal center, progressing centripetally to involve the foveal center and finally encompasses an oval area centered on the fovea, with a band of increased MP density along its boundaries.[15-17]

In optical coherence tomographic (OCT) images, low-reflecting spaces may be apparent in the inner and outer retina, while later in disease progressive atrophy of the outer retina occurs. Initially this is characterized by a discontinuity (break) in the line commonly attributed to the junctions of the photoreceptor inner and outer segments (or the ellipsoids of the inner segments, IS/OS line or ellipsoid zone, EZ),[18-20] with subsequent structural disorganization and apparent 'collapse' of the retinal layers internal to the IS/OS through the IS/OS break towards the retinal pigment epithelium (RPE).[18, 21, 22] The external limiting membrane (ELM), a thin hyper-reflective line adjacent to the EZ on OCT, is formed by tight junctions between the photoreceptors and Müller cells and its abnormalities may be considered a signal of structural changes in either cell type, its morphology in MacTel has however not been described. Histopathological studies have shown changes limited to the area delineated by BLR and structural changes in Müller cells.

The aim of the present study in search of clues to the pathogenesis of the disease and of signs permitting an early diagnosis, was to investigate the characteristics of abnormal retinal reflectivity to short-wavelength light and its relationship with other vascular and neurodegenerative clinical signs of MacTel detectable in multiple imaging modalities including autofluorescence, fluorescein angiographic, OCT and DWAF imaging.

PARTICIPANTS AND METHODS

Participants

Eyes of participants with a confirmed diagnosis from one site of the MacTel Study, an international multicenter prospective natural history observational study of MacTel[5] were chosen for this study. The site was selected on the basis of the availability of a wide range of imaging modalities including blue-light reflectance (BLR), autofluorescence (AF), fundus fluorescein angiographic (FFA), dual-wavelength autofluorescence (DWAF) and OCT images. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local institutional review board/ethics committee. Prior to enrolment in the study, written, informed consent was obtained from each participant following an explanation of the nature of the study.

Imaging

Images were recording in compliance with a standardised operating protocol by trained and certified image acquisition specialists. Blue light reflectance images were acquired using either a Heidelberg Retina Angiograph II or a Heidelberg Spectralis SLO system (Heidelberg Engineering GmbH, Heidelberg, Germany) using the 'red free' setting (utilizing a 488nm blue laser for illumination of the retina). Single-wavelength autofluorescence images were acquired using the same excitation wavelength, with a barrier filter for reflected light at <520nm. The distribution of macular pigment was recorded by dual-wavelength autofluorescence method using a Heidelberg Retina Angiograph Classic scanning laser ophthalmoscope system (Heidelberg Engineering GmbH, Heidelberg, Germany). This method has been described previously.[12-14] Briefly, two series of autofluorescence images of the fundus were recorded using monochromatic excitation lights of 488nm and 514nm wavelength respectively, through a barrier filter for returning light at <560nm. Individual images within each sequence were aligned using anatomic (mainly vascular) landmarks and averaged to improve the signal-to-noise ratio. A map of relative densities characteristic of macular pigment content was calculated from each corresponding pixel pair within the two averaged images. In our study 10-16 individual images were averaged at each wavelength prior to calculating the macular pigment density maps. Spectral domain OCT volume scans of the retina were acquired using Heidelberg Spectralis OCT systems (Heidelberg Engineering GmbH, Heidelberg, Germany). Raster volume scans covered a retinal area of at least 15x10 degrees in size, centered on the fovea, with a maximum B-scan interval of 30 microns. The transverse imaging module of the Heidelberg Eye Explorer (HEYEX) software was used for creating en face images of the EZ and ELM layers following manual inspection and where necessary correction of the automated segmentation of these specific layers provided by HEYEX.

Phenotyping

Images of all modalities (en face OCT of the EZ, of the ELM, early and late FFA, AF, DWAF and BLR were aligned to attain exact correspondence primarily using I2K Retina version 1.3.8 (DualAlign™ LLC) and collected in multi-layered PSD image files in Adobe Photoshop (version CS6, Adobe Systems Inc., San Jose, California, USA). The extent of the abnormality in the ELM was delineated based on the points of clear visibility of the ELM and both adjacent layers nearest to the retinal abnormality (typically a 'collapse') from to vantage points, the standard B-scan perspective and one at 90 to it (in the Y-Z plane) using a dedicated 3D imaging software package (Amira v5.3, FEI Inc, Hillsboro, Oregon, USA). Orthogonal maps (in the x-y, a.k.a. the 'en face' plane) of the boundary markers were

exported to Photoshop and aligned manually to the common geometry of the other imaging modalities. All image alignments were inspected and corrected manually as necessary in Adobe Photoshop. The extent of abnormalities in each imaging modality was delineated and measured (expressed in pixels) manually and their relative areas and locations within the plane of the retina were compared.

Due to their relatively low resolution and grainy quality, duplicates of layers containing DWAF images were created and a Gaussian filter was applied to reduce high frequency noise prior to delineation of the abnormal area. The distribution pattern of luteal pigment in healthy eyes demonstrates a peak at the foveal center and progressively decreases towards a plateau value in the peripheral retina. Concentration levels may vary, but the gradient is consistently towards the periphery. The boundary of the abnormal area in MacTel eyes was defined as the collection of points at which luteal pigment density starts to decline *towards* the foveal center.

The term 'MacTel area' (introduced by M. Fruttiger, unpublished communications) is defined as the region delimited by the outer boundary of increased reflectivity to short-wavelength light in well established disease (complete ellipse).

Grading and measurements were performed by one grader masked to the identity and clinical data of the patients. To reduce the risk of bias, delineation of lesion boundaries was performed in one imaging modality at a time across the whole available sample. Data necessary for calibration of true retinal image size (axial length and refractive power of the eye) were not available, however most analyses were limited to the relative lateral extent and topographic position of lesions within each eye.

Statistical Methods

The relationships of lesion extent in different imaging modalities was assessed by calculating Spearman's rank correlation coefficient. Spearman's ρ was calculated from grading data ranked in order of severity; calculations were based on the differences between the ranks of corresponding values of the independent and the dependent variable. The value of ρ may range between 1, indicating a perfect correlation and -1, indicating a perfect inverse (negative) correlation. A p value of <0.05 was accepted as statistically significant. All analyses were conducted using commercially available statistical software (SAS version 9.3; SAS Institute, Cary, NC, USA and MedCalc for Windows, version 12.5, MedCalc Software, Ostend, Belgium).

RESULTS

Full image sets of all imaging modalities were available of 106 eyes of 53 patients (32 women, 21 men), ranging in age between 30-82 years, (mean=61 years, SD=10.6 years) of a quality sufficient for a multimodal analysis.

In all uncomplicated MacTel cases, the BLR hyper-reflective pattern was confined to an oval area centered on the fovea, with the longest axis in the horizontal meridian (corresponding approximately to the parafoveal region), always presenting in the temporal part and covering varying degrees of the remaining area. The central foveal area (corresponding to the foveal pit, and in DWAF images to the central luteal pigment peak) appeared in BLR images dark at varying intensities. We measured the approximate horizontal and vertical diameters of the outer boundary of the BLR hyperreflective area in well-established cases (full ellipse), measurements are presented in Table 1.

Ten eyes of eight patients were excluded from the main analyses and analyzed separately due to very extensive lesions not directly attributable to the primary MacTel disease. In one eye a large area of subretinal fluid was present, which did not diminish either the BLR, DWAF or ELM signals; the visibility of the EZ was however impaired. In two eyes, a highly reflective epiretinal membrane (ERM) was present obscuring the boundary of the BLR pattern. The ERMs were constricting, causing retinal folds, the DWAF pattern was in both cases well detectable, although in one its shape was distorted. In six eyes of four patients a subretinal neovascular (NV) complex extending outside the MacTel area was present. Whereas small NV lesions do not seem to affect the visibility of either the BLR or DWAF signals, as the area size of the NV approaches that of the BLR/DWAF signal, first the BLR then the DWAF signals are attenuated or lost. The detectability of the DWAF signal was consistently better than that of the BLR signal, except in one case with subretinal hemorrhage larger than the parafoveal 'MacTel' area (from a small neovascular lesion), which improved the visibility of the BLR signal by providing a dark background, while obfuscating the DWAF signal.

In good quality BLR images, the area of increased reflectivity appeared to be present at variable intensities. In 30 eyes (28%) the whole “MacTel” area was evenly hyper-reflective. In 55 eyes, (52%) the high level BLR hyper-reflectivity was high temporally and superiorly but did not involve the full 'MacTel' area; in these a lower but clearly detectable level of increased reflectivity appeared to 'complete' the oval pattern (typically inferiorly or infero-nasally, see Figure 2 top panel, image A). Images in which this lower level was not visible (11 eyes, 10%) or questionable (14 eyes, 13%) were of low quality and the visibility of the main pattern was also consistently low. The central foveal area containing in DWAF images the central peak of luteal pigment density appeared in BLR images dark at variable levels.

The detectability of the pattern in BLR images was influenced by several factors, including obscuring lids and lashes, tear film anomalies (short break-up time, lipid droplets), crystalline lens and vitreous opacities. Obscuring technical factors included defocus, uneven field illumination and over-exposure. The pattern brightness appeared to be affected by directionality and to some extent by the type of SLO machine used (HRA or Spectralis). These phenomena may warrant a further systematic analysis. In this study however, the outcome measure was the lateral extent of the BLR hyperreflectivity (and not the intensity of the hyperreflectivity) and this was possible to measure in all images.

We assessed the inter-grader repeatability of measurements of the BLR hyperreflective area in well-established disease (complete ellipse) by calculating the intraclass correlation coefficient (ICC), investigating absolute agreement between two graders for all measurements, in a two-way model (n=18 eyes). The ICC was for single measures 0.9656

(95%CI 0.9065 to 0.9872) and for average measures 0.9825 (95%CI 0.9510 to 0.9936).

The detectability of the DWAF signal was consistently better than that of the BLR signal although this did not impede the analysis.

The concordance in area size of luteal pigment abnormality in DWAF images and the area of increased reflectivity to blue light (BLR) was excellent, for right eyes: ρ=0.917 ($p<0.0001$, 95%CI 0.855-0.954, n=46) and for left eyes: ρ=0.952 ($p<0.0001$, 95%CI 0.916-0.973, n=49). The mean (BLR) relative area of intersection was in right eyes 88% and in left eyes 87%. Removing neovascular cases from the analysis did not significantly affect the correlation.

The lateral extent of abnormalities in all other imaging modalities was smaller in area and located within the area occupied by the BLR/DWAF abnormality.

Single wavelength (blue) AF images provided a sharp boundary of a hyper-autofluorescence towards the foveal center (as well as very clear hypo-AF anomalies corresponding to brown pigment plaques in the mid-retina), however towards the periphery, the boundary was too indistinct to delineate in 28 of a total of 96 cases (29%).

The correlation between DWAF and AF lesion area was ρ=0.441 ($p<0.0091$, 95%CI 0.121-0.678, n=34) for right eyes and ρ=0.554 ($p<0.0009$, 95%CI 0.253-0.745, n=34) for left eyes. The AF abnormality occupied on average 53% of the DWAF abnormality in right eyes and 49% in left eyes.

The correlation between the BLR hyper-reflective area and the area of leakage/staining in the late-phase FFA was ρ=0.772 ($p<0.0001$, 95%CI 0.620-0.868, n=46) for right eyes and ρ=0.737 ($p<0.0001$, 95%CI 0.575-0.843, n=49) for left eyes. The FFA abnormality occupied on average 50% of the BLR hyper-reflective area in both right and left eyes.

The correlation between the BLR hyper-reflective area and the area of a break in the EZ (IS/OS layer) was ρ=0.631 ($p<0.0001$, 95%CI 0.418-0.779, n=46) for right eyes and ρ=0.593 ($p<0.0001$, 95%CI 0.374-0.749, n=49) for left eyes. The EZ (IS/OS) break extent was on average 15% of the BLR hyper-reflective area in right eyes and 12% in left eyes.

The correlation in area size and location of the ELM abnormality and the EZ (IS/OS) break was very good, ρ=0.989 ($p<0.0001$, 95%CI 0.980-0.994, n=46) for right eyes and ρ=0.978 ($p<0.0001$, 95%CI 0.961-0.987, n=50) for left eyes. In 32 of 46 right eyes, the area of the EZ break was larger than that of the the ELM abnormality (on average by 36% of the ELM area, median difference 18%), in 8 eyes it was smaller (on average by 10%, median 3%), in 6 eyes the two areas were the same. In 32 of 50 left eyes, the area of the EZ disruption was larger than that of the ELM lesion (by a mean 40%, median 14%), in 7 eyes it was smaller (on average by 5%, median 2%), in 11 eyes the two areas were the same. The magnitude of differences was affected by a few outliers (see scatter plot in supplemental Figure S1).

The characteristics and distribution of area measurements are shown in Table 2 and Figure 3. Agreement between right and left eyes is shown in Table 3. All changes were situated within the area delineated by DWAF and blue light reflectance (see supplemental Figure S2).

DISCUSSION

Type 2 idiopathic macular telangiectasia - as the name suggests - was long considered a primarily vascular disease. However, new imaging modalities including OCT, DWAF and BLR imaging have revealed numerous neurodegenerative signs of the disease and contributed to a paradigm shift in the hypothesis of its pathogenesis.

One of these neurodegenerative signs, a 'graying of the foveal area' was mentioned as a sign of parafoveal telangiectasia as early as 1978 by Gass et al.[23] This sign was later described as a 'loss of retinal transparency' and alone is the sole criterion of stage 2 in the severity classification of the disease devised by Gass and Blodi in 1993.[8] Charbel-Issa et al. demonstrated that this area exhibits an abnormally increased reflectivity to short wavelength (488nm) light and can be visualized best using a confocal scanning laser ophthalmoscope.[9, 10] Jindal et al. noted that the intensity appeared to fade with continuous light exposure and is restored following dark adaptation.[24] M. Fruttiger noted that all typical signs of MacTel appear to be located within an area defined by the outer boundary of increased reflectivity to blue light and suggested the term 'MacTel area' as occurred in our cases.

Our findings show that in the vast majority with early disease a well-defined area is apparent on the basis of hyper-reflectivity as seen on blue light reflection and a deficit of luteal pigment as recorded by dual wavelength autofluorescence. There was variation of intensity of reflectivity in the area as seen on blue light reflectance with the densest being in the temporal macula. It is well established that the phenomenon is intensified by dark adaptation[24]. Thus in those in which the area was not totally outlined, it is conceivable that it would have been complete following a short time in darkness. One limitation of our study is that the light exposure (bleaching) status of the eye at the time of BLR imaging was not recorded.

The area of abnormality as seen on single wavelength autofluorescence, and fluorescein angiography was limited to the temporal macula in early disease and expands to occupy the whole area as defined by blue light reflectance as the disorder progresses.[25] Deficits in the outer retinal lines as seen on OCT appear to occur later, starting temporal to the fovea and expands over time to fill the MacTel area. The progression of outer retinal changes becomes distorted in late disease by subretinal neovascularization with or without contraction by scar tissue in later disease. The progression of these changes appears to be sequentially from temporal to the fovea, to nasal, superior and lastly inferior to the fovea. Notable is the absence of extension outside the area defined early by blue light.

These findings are relevant to early diagnosis that is important in the quest for early diagnosis when attempting to generate pedigrees as is crucial for genetic studies. Dual wavelength autofluorescence or blue light reflectance appear to be equally sensitive. However, the former is not widely available such that blue light is the more useful. Important is the influence of dark adaptation on this finding[24] and if a clinician is in doubt as to whether or not the abnormality exists, placing the patient in the dark for 10 minutes or so might help in decision making.

These observations are also important to concepts as to the pathogenesis of disease. They indicate that abnormal metabolic handling of luteal pigment is widespread in the macula even in early disease. It has been shown that Müller cells are involved in the disease process[3, 4] and it is possible that Müller cells are involved in the metabolism of luteal pigments. There is evidence that Müller cell are involved in metabolism of other retinoids.[26]

It is also evident that the retinal region affected by disease is defined early in its progress by blue light refection and dual wavelength. Other changes follow to occupy the whole of that area but do not expand beyond it. This indicates that a specific susceptibility exits that is

limited to the MacTel area that presumably can be related to a metabolic attribute limited to this area. This may be related to the metabolic interdependence between Müller cells and photoreceptor cells.[27] That Müller cell dysfunction is associated with photoreceptor cell loss is well illustrated in rodents.[28, 29] Interestingly these rodents develop outer retinal vascularization consistently.[27]

The only clinically recognized entity that resembles MacTel is seen in Sjögren-Larsson syndrome.[25] Evidence has been sought of genetic similarity between the two disorders but none has been found. It is conceivable that certain forms Müller cell dysfunction may cause retinal disease that may be confined to the macula, regardless of the underlying cause.

REFERENCES

1. Gass JD, Oyakawa RT. Idiopathic juxtafoveolar retinal telangiectasis. Arch Ophthalmol 1982;100:769-80.
2. Charbel Issa P, Gillies MC, Chew EY, et al. Macular telangiectasia type 2. Prog Retin Eye Res 2013;34:49-77.
3. Powner MB, Gillies MC, Tretiach M, et al. Perifoveal muller cell depletion in a case of macular telangiectasia type 2. Ophthalmology 2010;117:2407-16.
4. Powner MB, Gillies MC, Zhu M, et al. Loss of Muller's cells and photoreceptors in macular telangiectasia type 2. Ophthalmology 2013;120:2344-52.
5. Clemons TE, Gillies MC, Chew EY, et al. Baseline characteristics of participants in the natural history study of macular telangiectasia (MacTel) MacTel Project Report No. 2. Ophthalmic Epidemiol;17:66-73.
6. Meleth AD, Toy BC, Nigam D, et al. Prevalence and progression of pigment clumping associated with idiopathic macular telangiectasia type 2. Retina 2013;33:762-70.
7. Sallo FB, Leung I, Chung M, et al. Retinal crystals in type 2 idiopathic macular telangiectasia. Ophthalmology 2011;118:2461-7.
8. Gass JD, Blodi BA. Idiopathic juxtafoveolar retinal telangiectasis. Update of classification and follow-up study. Ophthalmology 1993;100:1536-46.
9. Charbel Issa P, Finger RP, Helb HM, et al. A new diagnostic approach in patients with type 2 macular telangiectasia: confocal reflectance imaging. Acta Ophthalmol 2008;86:464-5.
10. Charbel Issa P, Berendschot TT, Staurenghi G, et al. Confocal blue reflectance imaging in type 2 idiopathic macular telangiectasia. Invest Ophthalmol Vis Sci 2008;49:1172-7.

11. Bottoni F, Eandi CM, Pedenovi S, Staurenghi G. Integrated clinical evaluation of Type 2A idiopathic juxtafoveolar retinal telangiectasis. Retina 2010;30:317-26.
12. Delori FC, Goger DG, Hammond BR, et al. Macular pigment density measured by autofluorescence spectrometry: comparison with reflectometry and heterochromatic flicker photometry. J Opt Soc Am A Opt Image Sci Vis 2001;18:1212-30.
13. Wustemeyer H, Jahn C, Nestler A, et al. A new instrument for the quantification of macular pigment density: first results in patients with AMD and healthy subjects. Graefes Arch Clin Exp Ophthalmol 2002;240:666-71.
14. 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;244:1565-74.
15. Helb HM, Charbel Issa P, RL VDV, et al. Abnormal macular pigment distribution in type 2 idiopathic macular telangiectasia. Retina 2008;28:808-16.
16. Zeimer MB, Padge B, Heimes B, Pauleikhoff D. Idiopathic macular telangiectasia type 2: distribution of macular pigment and functional investigations. Retina 2010;30:586-95.
17. Esposti SD, Egan C, Bunce C, et al. Macular Pigment Parameters in Patients with Macular Telangiectasia (MacTel) and Normal Subjects: Implications of a Novel Analysis. Invest Ophthalmol Vis Sci 2012;53:6568-75.
18. Gaudric A, Ducos de Lahitte G, Cohen SY, et al. Optical coherence tomography in group 2A idiopathic juxtafoveolar retinal telangiectasis. Arch Ophthalmol 2006;124:1410-9.
19. Yannuzzi LA, Bardal AM, Freund KB, et al. Idiopathic macular telangiectasia. Arch Ophthalmol 2006;124:450-60.

20. Maruko I, Iida T, Sekiryu T, Fujiwara T. Early morphological changes and functional abnormalities in group 2A idiopathic juxtafoveolar retinal telangiectasis using spectral domain optical coherence tomography and microperimetry. Br J Ophthalmol 2008;92:1488-91.
21. Sallo FB, Peto T, Egan C, et al. The IS/OS junction layer in the natural history of Type 2 Idiopathic Macular Telangiectasia. Invest Ophthalmol Vis Sci 2012.
22. Sallo FB, Peto T, Egan C, et al. "En face" OCT Imaging of the IS/OS Junction Line in Type 2 Idiopathic Macular Telangiectasia. Invest Ophthalmol Vis Sci 2012;53:6145-52.
23. Gass JDM. Stereoscopic atlas of macular diseases : diagnosis and treatment, 2d ed. St. Louis: C. V. Mosby, 1977; xi, 411 p.
24. Jindal A, Choudhury H, Pathengay A, Flynn HW, Jr. A novel clinical sign in macular telangiectasia type 2. Ophthalmic Surg Lasers Imaging Retina 2015;46:134-6.
25. Theelen T, Berendschot TT, Klevering BJ, et al. Multimodal imaging of the macula in hereditary and acquired lack of macular pigment. Acta Ophthalmol 2014;92:138-42.
26. Bunt-Milam AH, Saari JC. Immunocytochemical localization of two retinoid-binding proteins in vertebrate retina. J Cell Biol 1983;97:703-12.
27. Lindsay KJ, Du J, Sloat SR, et al. Pyruvate kinase and aspartate-glutamate carrier distributions reveal key metabolic links between neurons and glia in retina. Proc Natl Acad Sci U S A 2014;111:15579-84.
28. Dudok JJ, Sanz AS, Lundvig DM, et al. MPP3 regulates levels of PALS1 and adhesion between photoreceptors and Muller cells. Glia 2013;61:1629-44.
29. Zhao M, Andrieu-Soler C, Kowalczuk L, et al. A new CRB1 rat mutation links Muller glial cells to retinal telangiectasia. J Neurosci 2015;35:6093-106.

FIGURE LEGENDS

Figure 1. Aligned multimodal images of a MacTel eye with very early disease.
A: 488nm (blue) reflectance image, **B:** dual-wavelength autofluorescence image (DWAF), **C:** single wavelength (488nm) fundus autofluorescence image, **D:** infrared image, **E** and **F:** early and late phase fluorescein angiograms, **G:** IR image with the en face view of the Ellipsoid Zone superimposed, **H:** IR image with the en face map of the External Limiting Membrane (ELM) superimposed. An eye with very early mactel disease from an asymmetrical case, demonstrating essentially no sign of mactel and only a few diabetes-related microaneurysms in the FFA. The FAF, DWAF images appear essentially normal, demonstrating the peak of luteal pigment density at the foveal center, that decreases towards the periphery. The dark central area in the EZ map corresponds to the rod-free center of the fovea externa with very high packing density of cones, where the EZ signal is normally weaker. The fellow eye (not shown) demonstrates all typical signs of MacTel.

Figure 2. BLR imaging in MacTel and multimodal correlates.
Aligned multimodal images of a relatively early case (top panel) and a later-stage MacTel eye, bottom panel. A: blue light reflectance images, B: dual wavelength (488+514nm) autofluorescence images, C: single (488nm, blue) autofluorescence images, D: early phase FFA images, E: late-phase FFA images, F: topographic maps of the EZ (IS/OS layer). The small, round, hyper-reflective area close to the fovea in the BLR image in the top panel is an artifact due to internal reflections within the lens system of the Spectralis SLO system.

Figure 3. Correlation of lesion extent in multimodal images of type 2 MacTel
Top row: BLR vs DWAF area in (A) right and (B) in left eyes. Second row: DWAF area vs single wavelength blue light AF area, in right (C) and in left eyes (D). NB: in 28/96 eyes the AF lesion was not possible to delineate due to an indistinct peripheral boundary. Third row: BLR hyper-reflective area vs late FFA area, in right (E) and in left eyes (F). Bottom row: BLR hyper-reflective area vs EZ (IS/OS) break area, in right (G) and in left eyes (H). All data shown are expressed in x1000 pixels (proprietary units).

Online-only supplemental information:

Figure S1. The correlation between EZ and ELM lesion area.
Scatter plot indicating the relationship of the area size of the abnormality in transverse (en face) images of the Ellipsoid Zone (EZ) and the External Limiting Membrane (ELM) in each eye in the study (left and right eyes, n=106). The ellipsoid zone is considered a photoreceptor signal (emanating from the inner segment ellipsoids or the junctions between the inner and outer segments) whereas the ELM has its source in the junctions between the photoreceptors and Müller cells. The ELM may be considered a Müller cell signal.

Figure S2. The extent of abnormalities in different imaging modalities relative to BLR.
This image set is the same as the top panel in Figure 2, but with a dotted line marking the outer boundary of the hyper-reflective area in BLR imaging superimposed. In MacTel, the extent of abnormalities in BLR (**A**) and DWAF (**B**) images share this boundary, whereas abnormalities in all other imaging modalities (C-H) are located well within it. **A:** 488nm (blue) reflectance (BLR) image, **B:** dual wavelenght autofluorescence image (DWAF), **C:** single wavelength (488nm) fundus autofluorescence image, **D:** infrared image, **E** and **F:** early and late phase fluorescein angiograms, **G:** IR image with the en face view of the Ellipsoid Zone superimposed, **H:** IR image with the en face map of the External Limiting Membrane (ELM) superimposed.

APPENDIX

Participating Principal Investigators and Centers in the MacTel Study:

Jose-Alain Sahel, MD, PhD, Centre Hôpitalier National D'Ophtalmologie des Quinze-Vingts, Paris, France;
Robyn Guymer, MD, Center for Eye Research, East Melbourne, Australia;
Gisèle Soubrane, MD, PhD, FEBO, Clinique Ophtalmologie de Créteil, Créteil, France;
Alain Gaudric, MD, Hôpital Lariboisière, Paris, France;
Jean-Pierre Hubschman, MD, Steven Schwartz, MD, Jules Stein Eye Institute, UCLA, Los Angeles, CA (USA);
Ian Constable, MD, Lions Eye Institute, Nedlands, Australia;
Michael Cooney, MD, MBA, Manhattan Eye, Ear, & Throat Hospital, New York, NY (USA);
Catherine Egan, MD, Moorfields Eye Hospital, London, England (UK);
Lawrence Singerman, MD, Retina Associates of Cleveland, Cleveland, OH (USA);
Mark C Gillies, MD, PhD, Save Sight Institute, Sydney, Australia;
Martin Friedlander, MD, PhD, Scripps Research Institute, La Jolla, CA (USA);
Daniel Pauleikhoff, Prof. Dr., St. Franziskus Hospital, Muenster, Germany;
Joseph Moisseiev, MD, Goldschleger Eye Institute, Tel Hashomer, Israel;
Richard Rosen, MD, New York Eye and Ear Infirmary, New York, NY (USA);
Robert Murphy, MD, Retina Group of Washington, Fairfax, VA (USA);
Frank Holz, MD, University of Bonn, Bonn Germany;
Grant Comer, MD, University of Michigan, Kellogg Eye Center, Ann Arbor, MI (USA);
Barbara Blodi, MD, University of Wisconsin, Madison, WI (USA);
Diana Do, MD, Wilmer Eye Institute, Baltimore, MD (USA);
Alexander Brucker, MD, Scheie Eye Institute, Philadelphia, PA (USA);
Raja Narayanan, MD, LV Prasad Eye Institute, Hyderabad, India;
Sebastian Wolf, MD, PhD, University of Bern, Bern, Switzerland;
Philip Rosenfeld, MD, PhD, Bascom Palmer, Miami, FL (USA).
Paul S Bernstein, MD, PhD, Moran Eye Center, University of Utah, UT (USA)
Joan W Miller, MD, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA (USA)
Lawrence Yannuzzi, MD, Vitreous, Retina, Macula Consultants of New York, NY, (USA)
Jacque Duncan, MD, University of California, San Francisco, CA, (USA)
Mina Chung, MD, University of Rochester Medical Center, Rochester, NY, (USA)
Jiong Yan, MD, Emory University, Atlanta, GA, (USA)
David Weinberg, MD, Medical College of Wisconsin, Milwaukee, WI, (USA)
Clasien Oomen, MD, Radboud University Medical Center, Nijmegen, Holland