AMERICAN ACADEMY OF OPHTHALMOLOGY®

Fluorescence Lifetime Imaging Ophthalmoscopy: A Novel Way to Assess Macular Telangiectasia Type 2

Lydia Sauer, MD,[1,2] *Rebekah H. Gensure, MD, PhD,*[1] *Martin Hammer, PhD,*[2] *Paul S. Bernstein, MD, PhD*[1]

Purpose: Macular telangiectasia type 2 (MacTel) is an uncommon, late-onset complex retinal disease that leads to central vision loss. No causative gene(s) have been identified to date, resulting in a challenging clinical diagnostic dilemma because retinal changes of early stages are often subtle. The objective of this study was to investigate the benefit of fluorescence lifetime imaging ophthalmoscopy (FLIO) for retinal imaging in patients with MacTel.

Design: Cross-sectional study from a tertiary care retinal referral practice.

Participants: Both eyes of 21 patients (mean age 60.5±13.3 years) with MacTel as well as an age-matched healthy control group (42 eyes of 25 subjects, mean age 60.8±13.4 years).

Methods: A 30° retinal field centered at the fovea was investigated using FLIO. This camera is based on a Heidelberg Engineering Spectralis system (Heidelberg Engineering, Heidelberg, Germany). Fundus autofluorescence (FAF) decays were detected in both short spectral channels (SSC, 498−560 nm) and long spectral channels (LSC, 560−720 nm). The mean fluorescence lifetime, τ_m, was calculated from a 3-exponential approximation of the FAF decays. For patients with MacTel, macular pigment (MP), OCT, blue-light reflectance, fluorescein angiography, and fundus photography were also recorded.

Main Outcome Measures: Mean FAF lifetime (τ_m) images.

Results: FLIO of patients with MacTel shows a unique pattern of prolonged τ_m at the temporal side of the fovea in patients with MacTel in the "MacTel area" within 5° to 6° of the foveal center. In early stages, this region appears crescent-shaped, whereas advanced stages show a ringlike pattern. This pattern corresponds well with other imaging modalities and provides especially high contrast of the affected region even in minimally affected individuals. Additionally, FLIO provides a novel means to monitor the abnormal MP distribution. In one case, FLIO showed changes suggestive of MacTel within a clinically normal parent of two patients with MacTel.

Conclusions: FLIO detects retinal changes in patients with MacTel with high contrast, presenting a distinctive signature that is a characteristic finding of the disease. The noninvasive properties of this novel imaging modality provide a valuable addition to clinical assessment of early changes in the disease that could lead to more accurate diagnosis of MacTel. *Ophthalmology Retina 2017;■:1−12 © 2017 by the American Academy of Ophthalmology*

Macular telangiectasia type 2 (MacTel) is an uncommon hereditary disease that gradually results in vascular and neurodegenerative changes, leading to metamorphopsia and central vision loss.[1] Whereas macular telangiectasia type 1 is very rare and manifests typically as a unilateral disease in young males, type 2 is a bilateral disease with a later onset at 40 to 60 years of age.[2−4] Patients affected by MacTel have low scores on National Eye Institute Visual Function Questionnaires.[5] A prevalence of 0.06% to 0.1% has been reported; however, it is believed that this number is underestimated.[6] Because early diagnosis is difficult, patients may be misdiagnosed with age-related macular degeneration (AMD) instead.[7] The underlying pathogenesis of the disease is still unknown; however, a dominant genetic inheritance with reduced penetrance is likely.[8−10] Recently two genetic loci within the glycine/serine pathway were identified for MacTel, but the actual genes related to the disease have not yet been found; thus genetic testing is not yet available.[8,11,12] It has been reported that a loss of Müller and photoreceptor cells plays a key role in the development of the disease.[13,14] The loss of the ellipsoid zone typically starts temporal to the foveal center and progresses in all directions and may eventually involve the entire MacTel area. This pattern is associated with a loss of retinal sensitivity and eventual loss of visual acuity once the degeneration reaches the foveal center.[15,16] Additionally, an abnormal distribution of macular pigment (MP) at 5° to 9° eccentricity has been described.[17−21] Macular pigment levels are generally low, yet may appear normal in early stages of the disease.[22] Although changes in MP binding proteins do not appear to be associated with a loss of Müller cells, supplementation with carotenoids does not reestablish the typical MP distribution but rather causes a ringlike enhancement of MP outside the fovea.[19,23,24]

https://doi.org/10.1016/j.oret.2017.10.008
ISSN 2468-6530/17

Other findings in MacTel include intraretinal crystals in approximately 46% of patients, which typically appear at the level of the inner limiting membrane at all stages of the disease. The origin of these crystals remains unclear.[25–28]

Presently there are no treatments for MacTel. Neither intravitreal steroid nor antivascular endothelial growth factor injections have been promising and seem to have no effect on improving visual outcome.[29–31] The effect of ciliary neurotrophic factor may be beneficial and is currently under investigation.[33]

Multiple imaging modalities show unique changes within MacTel, including color fundus imaging, fundus autofluorescence (FAF) intensity imaging, OCT, fluorescein angiography, and blue-light reflectance imaging.[34–37] Fluorescein angiography has historically been used as the gold standard in imaging for MacTel; however, recently other noninvasive imaging modalities have been proposed as useful.[29,34,38–40] In patients with MacTel the disease can be diagnosed when retinal damage is visible, but diagnosis at early stages is still challenging. At this point, there is no clear characteristic set of findings in the early course of MacTel, leading in many cases to delayed diagnosis. A lack of the foveal reflex and retinal graying are often the first clinical signs. Mildly ectatic capillaries in the deeper capillary network and dilated venules may develop later in the course of the disease. Retinal pigment clumps and foveal atrophy are often found in late stages of the disease.[28] Retinal alterations often start at the temporal paracentral side of the fovea.[41] They may eventually affect the entire so-called MacTel area, which has been described as an oval region with approximately 5° vertical and 6° horizontal eccentricities. This is the maximal area clinically affected in eyes with advanced MacTel.[42–44] Increased central autofluorescence may be associated with poorer central visual acuity.[45]

Fluorescence lifetime imaging ophthalmoscopy (FLIO) is a new imaging technology that offers a novel approach for early diagnosis of various retinal diseases and reproducibly provides additional information on the autofluorescence of the retina.[46,47] Detecting FAF lifetimes allows the detection of subtle changes within retinal molecules at early stages of diseases. Molecular changes alter the FAF lifetimes, leading to different patterns within the retina.[46,48–54] These molecular changes may be an early predictor of retinal changes even before retinal damage is visible to an ophthalmologist. Additionally, FLIO depicts changes in MP in the course of various diseases, such as macular holes.[55,56] The high contrast with which FLIO highlights changes in the retina could lead to improved clinical detection of MacTel. This study investigates the potential benefit of FLIO imaging in patients with MacTel.

Methods

Subjects

This prospective, cross-sectional study was approved by the institutional review board of the University of Utah, Salt Lake City, UT and adhered to the Declaration of Helsinki. The study is compliant with the Health Insurance Portability and Accountability Act of 1996. Informed written consent was obtained from the patients before all investigations.

All patients and healthy controls were examined between March and May 2017 and recruited from the Moran Eye Center.

Procedure

An ophthalmologist examined and diagnosed all patients with MacTel with confirmation by the Moorfields Eye Hospital Reading Center. Optical coherence tomography, blue-light reflectance, fluorescein angiography, and dual-wavelength autofluorescence images were made available to the reading center. The central best corrected visual acuity was obtained. The intraocular pressure was measured with a Tono-Pen (Reichert Technologies, Buffalo, NY) and pupils were dilated.

After 30 minutes, a noninvasive FLIO measurement was performed with an acquisition time of approximately 2 minutes per eye. A frequency domain OCT image and MP levels were also obtained. Macular pigment was measured using dual-wavelength autofluorescence imaging. Fundus photography, blue-light reflectance, and fluorescein angiography were recorded as well.

FLIO Setup and Image Acquisition

Based on existing Heidelberg Engineering Spectralis technology, FLIO records FAF lifetimes from a 30° retinal field in vivo and relies on the principle of time-correlated single photon counting.[57,58] The detailed setup and safety of FLIO have been described previously.[47,55,57] Briefly, FAF is excited by a pulsed diode laser (473 nm, 80 MHz). Two hybrid photomultipliers (HPM-100-40; Becker & Hickl GMBH, Berlin, Germany) detect fluorescence photons, resulting in two separate spectral channels: the short spectral channel (SSC, 498–560 nm) and the long spectral channel (LSC, 560–720 nm). A high-contrast confocal infrared reflectance image for eye tracking is included. A photon arrival histogram, representing the probability density function of the decay process, is based on the detection of photons into 1024 time channels. To ensure reliable image quality, at least 1000 photons were recorded for each pixel as a minimal signal threshold. All patients had intraocular pressures tested without the use of topical fluorescein, and FLIO was always performed prior to any fluorescein angiography.

The fluorescence data were analyzed using SPCImage 4.4.2 software (Becker & Hickl). The fluorescence decay was approximated by calculating the least-square fit of a series of three exponential functions. A 3 × 3-pixel binning was used for noise reduction. The amplitude-weighted mean fluorescence decay time, τ_m, was used for further analysis, representing the amplitude-weighted average of the three time constants from the fit. Further details have been described elsewhere.[55,58]

FLIMX software was used for all FAF lifetime analyses and to generate pseudo-color images of the FAF lifetimes.[59] This software is documented and freely available for download online under the open-source Berkeley Source Distribution (BSD) license (http://www.flimx.de). A standardized Early Treatment Diabetic Retinopathy Study (ETDRS) grid was applied to obtain mean FAF lifetimes from different regions of interest (ROIs): C (central fovea), T1 (inner temporal region), N1 (inner nasal region), and T2 (outer temporal region). Image Processing and Analysis in Java (ImageJ, National Institutes of Health) was used to show the 3-dimensional distribution of MP measured with the dual-wavelength autofluorescence method.

Statistical Analysis

SPSS 21 (SPSS Inc, Chicago, IL) was used for all statistical analyses. A *t* test for paired samples was used to test for significant τ_m