Evaluation of Retinal Pigment Epithelium Layer Change in Vogt-Koyanagi-Harada Disease With Multicontrast Optical Coherence Tomography

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PURPOSE. Clinical evaluation of retinal pigment epithelium (RPE) change is important for the therapeutic management of chronic Vogt-Koyanagi-Harada (VKH) disease. We evaluated long-term change in the RPE layer in VKH disease, using near-infrared (NIR; 817 nm) images and autofluorescence images at 488 nm (short-wavelength [SW]-AF) and 785 nm (NIR-AF), and compared those images with images from multicontrast optical coherence tomography (MC-OCT). MC-OCT is capable of simultaneous measurement of OCT angiography, polarization-sensitive OCT, and standard OCT.

METHODS. We evaluated 24 eyes of 12 patients with chronic VKH disease. RPE changes were assessed using NIR, NIR-AF, SW-AF, and MC-OCT imaging performed from 6 to 48 months after disease onset. RPE-melanin-specific contrast OCT images were calculated using the dataset from MC-OCT.

RESULTS. Granular hyper NIR-AF lesions were observed in 8 of 24 eyes (33%). Eyes with granular hyper NIR-AF lesions showed a sunset glow fundus appearance significantly more frequently than did eyes without such lesions (P < 0.0001). MC-OCT imaging confirmed that there was melanin accumulation at the RPE-Bruch’s membrane band at the location of granular hyper NIR-AF lesions. Granular hyper NIR-AF lesions were clear in SW-AF and color fundus images, but clearly detectable in NIR images. Areas of hyper NIR-AF lesions gradually decreased over time.

CONCLUSIONS. Melanin accumulation in the RPE layer at the location of granular hyper NIR-AF lesions was confirmed with MC-OCT imaging. Long-term follow-up showed the reversible nature of this accumulation. MC-OCT is useful for the evaluation of change at the RPE layer in chronic VKH disease.

Keywords: melanin, optical coherence tomography, vogt-koyanagi-harada disease, polarization, retinal pigment epithelium

Vogt-Koyanagi-Harada (VKH) disease is an immune-mediated disorder that affects melanocyte-rich organs including the eye, skin, meninges, and inner ear.1 The acute uveitic stage of VKH disease is characterized by multiple fluid leakages at the level of the retinal pigment epithelium (RPE), with bilateral exudative retinal detachments.1 In the chronic stage, depigmentation or hyperpigmentation changes of the RPE may occur,2,3 which can lead to vision-threatening complications, including choroidal neovascularization, subretinal fibrosis, and chorioretinal atrophy.1,3,4 Therefore, clinical evaluation of the RPE changes is important for a better understanding of the therapeutic management of chronic VKH disease.

Clinical autofluorescence (AF) imaging is widely used to evaluate RPE changes in various retinal disorders. Hyper-AF in short-wavelength AF (SW-AF) imaging at 480 nm is thought to derive from lipofuscin or melanolipofuscin in the RPE,5,6 or from sheared photoreceptor outer segments.7 Hyper-AF in near-infrared AF (NIR-AF) imaging at 780 nm is thought to originate from melanin or melanolipofuscin in the RPE, from choroidal melanocytes, or from melanin-laden inflammatory cells.8,9 In chronic VKH disease, the presence of hyper-AF lesions, and its possible association with focal thickening of the RPE-Bruch’s membrane band10,11 or damage in the outer retinal layer,12 has been reported. NIR-AF imaging with adaptive optics could provide cellular-level imaging and might be useful for evaluating RPE cells.13 Although AF imaging provides important information about RPE changes, the absence of topographic information remains an important limitation of this technique. Moreover, a lack of specific contrast in RPE cells in intensity-based OCT images (so-called standard OCT images) impedes...
the direct comparison of standard OCT imaging and AF imaging. Polarization-sensitive OCT (PS-OCT) is a functional extension of OCT technology that measures the polarization properties of the eye.\textsuperscript{14,15} Melanin in tissues, including the RPE, can scatter light, causing depolarization in PS-OCT imaging.\textsuperscript{16} PS-OCT can provide three-dimensional (3D) melanin-specific contrast images by measuring depolarization in the retina and choroid.\textsuperscript{14,15,17} Our group previously evaluated chronic VKH disease with PS-OCT and demonstrated that the disappearance of choroidal melanin, resulting in a sunset glow fundus appearance, could be objectively evaluated with PS-OCT.\textsuperscript{17} Comprehensive evaluation of RPE changes in age-related macular degeneration could be performed with a combination of PS-OCT and NIR-AF imaging.\textsuperscript{18} However, the evaluation of melanin in RPE (RPE-melanin) with PS-OCT has some limitations, due to the inability to discriminate between melanin in the RPE and that in the choroid.\textsuperscript{19} Discrimination of RPE-melanin in PS-OCT imaging is important for the proper evaluation of RPE changes in chronic VKH disease.

To overcome this limitation, we developed a prototype multicontrast OCT (MC-OCT) system,\textsuperscript{20} which is capable of the simultaneous measurement of OCT angiography, PS-OCT, and standard OCT. From the dataset of MC-OCT, a pixel-wise segmentation method for RPE-melanin was developed and used to create RPE-melanin-specific contrast images to evaluate RPE-melanin changes.\textsuperscript{21} In our previous study, in patients with age-related macular degeneration, the similarity between NIR-AF images and en face projections of RPE-melanin-specific contrast OCT images was confirmed.\textsuperscript{21} Therefore, RPE-melanin-specific contrast OCT imaging might be useful for evaluating RPE change in VKH disease. In this study, we performed a long-term follow-up study with AF and MC-OCT imaging, and evaluated long-term RPE changes in chronic VKH disease.

**Methods**

**Subjects**

This prospective, observational, cross-sectional study was performed using a protocol that adheres to the tenets of the Declaration of Helsinki, and Institutional Review Board approval was obtained from the Tokyo Medical University (IRB 16-15). The study was registered in a public database (UMIN000026307; http://www.umin.ac.jp/ctr/index-j.htm). The nature of the current study and the implications of participating in this research were explained to all study candidates, and written informed consent was obtained from each participant before any study procedures or examinations were performed.

We examined 24 eyes from 12 Japanese patients (5 males, 7 females) with chronic VKH disease (Table). The mean age at onset of VKH disease was 57.1 years (range, 36–80 years). The diagnosis of VKH disease was based on the revised criteria proposed by the International Nomenclature Committee.\textsuperscript{22} Within the patient cohort, 6 eyes from 3 patients were in the chronic/recurrence phase, and the other 18 eyes were in the convalescent phase without recurrence. All subjects were initially treated with 6 to 10 mg intravenous betamethasone for 3 to 13 days, followed by oral prednisolone. Eyes with severe cataract or other eye diseases that could compromise the image quality were excluded.

**Multicontrast Optical Coherence Tomography (MC-OCT)**

Fully functional MC-OCT\textsuperscript{20} and its simplified version (simplified MC-OCT)\textsuperscript{23} were used to evaluate RPE-melanin changes. Fully functional MC-OCT provides standard OCT, OCT angiography, degree of polarization uniformity (DOPU),\textsuperscript{24} and birefringence. Simplified MC-OCT uses simpler hardware; hence it is more compact and stable than the fully functional MC-OCT. Simplified MC-OCT provides all the types of images that can be measured with fully functional MC-OCT except birefringence. Since birefringence was not used in this study, these two systems could be regarded as identical. Fully functional MC-OCT was used for the measurements at 6 months after onset, and simplified MC-OCT was used at 48 months after onset. These MC-OCT systems used a swept-source laser with a central wavelength of 1.05 μm, and their axial scan speed was 100,000 A-scans/s. A horizontal-fast raster scanning protocol, with 512 A-lines × 256 B-scans covering a 6.0 × 6.0-mm region on the retina, was used for volumetric scans. B-scan measurements were repeated four times at a single location. MC-OCT volumes without significant motion artifacts were used for this study. The MC-OCT systems compute multiple contrasts from a single dataset, including standard OCT, PS-OCT, and OCT angiography. Standard B-scan OCT images were obtained by coherent composition of four repetitive B-scans. OCT angiography was calculated using the complex Jones matrix correlation method with noise correction.\textsuperscript{25} The DOPU\textsuperscript{24} was calculated with Makita’s noise correction\textsuperscript{26} and computed using a 3 pixel (transverse) × 3-pixel (depth) kernel. The presence of low DOPU indicates

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**Table. Summary of Patients With Vogt-Koyanagi-Harada Disease**

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age at Onset, y</th>
<th>Hyper NIR-AF Lesions</th>
<th>Sunet Glow Fundus</th>
<th>Phase of VKH Disease</th>
<th>Initial Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 F</td>
<td>44</td>
<td>Granular, placoid</td>
<td>Granular, placoid</td>
<td>Without sunset</td>
<td>Convalescent phase</td>
<td>BTM 6 mg × 3 d</td>
</tr>
<tr>
<td>2 M</td>
<td>52</td>
<td>Granular</td>
<td>Granular</td>
<td>Sunset</td>
<td>Convalescent phase</td>
<td>BTM 8 mg × 8 d</td>
</tr>
<tr>
<td>3 M</td>
<td>64</td>
<td>Granular</td>
<td>Granular</td>
<td>Sunset</td>
<td>Chronic/recurrence phase</td>
<td>BTM 8 mg × 8 d</td>
</tr>
<tr>
<td>4 M</td>
<td>54</td>
<td>Granular</td>
<td>Granular</td>
<td>Without sunset</td>
<td>Chronic/recurrence phase</td>
<td>BTM 8 mg × 13 d</td>
</tr>
<tr>
<td>5 F</td>
<td>67</td>
<td>None</td>
<td>None</td>
<td>Without sunset</td>
<td>Convalescent phase</td>
<td>BTM 8 mg × 12 d</td>
</tr>
<tr>
<td>6 F</td>
<td>41</td>
<td>None</td>
<td>None</td>
<td>Without sunset</td>
<td>Convalescent phase</td>
<td>BTM 8 mg × 7 d</td>
</tr>
<tr>
<td>7 F</td>
<td>49</td>
<td>None</td>
<td>None</td>
<td>Without sunset</td>
<td>Convalescent phase</td>
<td>BTM 8 mg × 7 d</td>
</tr>
<tr>
<td>8 F</td>
<td>80</td>
<td>None</td>
<td>None</td>
<td>Without sunset</td>
<td>Convalescent phase</td>
<td>BTM 8 mg × 8 d</td>
</tr>
<tr>
<td>9 F</td>
<td>36</td>
<td>None</td>
<td>None</td>
<td>Without sunset</td>
<td>Convalescent phase</td>
<td>BTM 8 mg × 8 d</td>
</tr>
<tr>
<td>10 F</td>
<td>69</td>
<td>None</td>
<td>None</td>
<td>Without sunset</td>
<td>Convalescent phase</td>
<td>BTM 8 mg × 8 d</td>
</tr>
<tr>
<td>11 M</td>
<td>68</td>
<td>None</td>
<td>None</td>
<td>Without sunset</td>
<td>Convalescent phase</td>
<td>BTM 8 mg × 10 d</td>
</tr>
<tr>
<td>12 M</td>
<td>61</td>
<td>None</td>
<td>None</td>
<td>Without sunset</td>
<td>Convalescent phase</td>
<td>BTM 8 mg × 7 d</td>
</tr>
</tbody>
</table>

F, female; M, male; BTM, betamethasone.
depolarization by multiple scattered lights from melanosomes. A chorioretinal melanin thickness map was created by counting the number of pixels with low DOPU (<0.8) at each A-line in the 3D dataset. A chorioretinal melanin thickness map represents the overall thickness of melanin in the choroid and RPE.

For automatic segmentation of RPE-melanin, we computed a new index ($F_{\text{RPE}}$) using an attenuation coefficient, the DOPU, and the blood flow signal in OCT angiography, as

$$F_{\text{RPE}} = \frac{\text{attenuation coefficient} \times (1 - \text{DOPU}) \times (1 - \text{OCTA}_b)}{\text{OCTA}_b}.$$  

where OCTA is the binarized OCT angiography signal. This index was computed to selectively highlight only that melanin associated with the RPE. Melanin in both the RPE and choroid showed low DOPU. However, the blood flow signal in OCT angiography of the RPE-melanin layer was low due to the absence of vascularization, whereas the choroid showed a high blood flow signal due to dense vasculature. Hence the $F_{\text{RPE}}$ assumes a large value only for the RPE melanin.

The RPE-melanin cross-sectional images that represent the distribution of $F_{\text{RPE}}$ in the B-scan images were generated to evaluate the depth-resolved distribution of RPE-melanin. RPE-melanin thickness maps were created by counting the number of pixels with high $F_{\text{RPE}} (>0.15)$ at each A-line in the 3D dataset. An RPE-melanin thickness map represents the en face distribution of the thickness of RPE-melanin.

**Multimodal Imaging**

For multimodal imaging, NIR images (817 nm), NIR-AF images (785-nm excitation, emission > 800 nm), and SW-AF images (488-nm excitation, emission > 500 nm) were obtained with a Heidelberg Retina Angiograph 2 (Heidelberg Engineering, Heidelberg, Germany), and square images with side lengths of 30° (768 × 768 pixels) were saved in 8-bit grayscale.

To evaluate the alteration of granular hyper NIR-AF lesions over time, series of NIR-AF images were manually aligned, using retinal vascular architecture, with image-processing software (Adobe Photoshop CS5; Adobe Systems, San Jose, CA, USA). Magnification of the NIR-AF images was measured with the built-in program on the Heidelberg Retina Angiograph 2, and square images with side lengths of 3 mm were used for the measurement of hyper NIR-AF areas. In these areas, hyper NIR-AF lesions consisted solely of granular hyper NIR-AF lesions, without placoid hyper NIR-AF lesions. After application of a bandpass filter, using open-source image-processing software (Fiji; http://fiji.sc), the margin of the largest hyper NIR-AF area was manually delineated (Fig. 1). Mean luminance and standard deviation of the luminance of the largest hyper NIR-AF area were measured to calculate the threshold ($T_{\text{NIRAF}}$):

$$T_{\text{NIRAF}} = \frac{\text{mean luminance} \times \text{standard deviation of luminance}}{\text{OCTA}_b}.$$  

$T_{\text{NIRAF}}$ was used to binarize the images (Fig. 1), to measure the area of hyper NIR-AF with image-processing software.
FIGURE 2. Multimodal imaging of the right eye of a 44-year-old female (case 1) at 6 months after disease onset. Color fundus image (A) shows faint hyperpigmentation in the macula (white arrow). The NIR-AF image (B) shows granular hyper-AF lesions (white arrow), a placoid hyper-AF lesion (yellow arrow), and granular hypo-AF lesions (red arrow). The white line designates the scanning multicontrast optical coherence tomography line in (E–G), and the yellow line designates the scanning line in (K–M). The NIR image (C) shows high reflectivity at the location of granular hyper NIR-AF lesions. The short-wavelength AF image (D) shows indistinct hyper-AF at the granular hyper NIR-AF lesions (white arrow) and clear hyper-AF at the placoid hyper NIR-AF lesion (yellow arrow). The white square in the standard OCT B-scan image (E) indicates the region that is shown in the high-magnification image (F). Standard OCT B-scan images (E, F) show focal thickening of the retinal pigment epithelium (RPE)-Bruch’s membrane band (white arrow) and the presence of choroidal melanin (red arrow). A degree of polarization uniformity B-scan image (G) shows melanin accumulation at the RPE-Bruch’s membrane band (white arrow) and the presence of choroidal melanin (red arrow). Changes in the RPE are not clearly visible in the chorioretinal melanin thickness map (H). The RPE-melanin cross-sectional image (I) and RPE-melanin thickness map (J) illustrate RPE-melanin accumulation at the granular hyper NIR-AF lesions (white arrows). The region marked by the white square in the standard OCT B-scan image (K) is shown in the high-magnification image (L). Standard OCT B-scan images (K, L) show outer retinal damage (L) (white arrow) at the placoid hyper NIR-AF lesion, without particular findings in RPE-melanin in the RPE-melanin cross-sectional image (M). Scale bar: 500 × 500 μm.
Then, the rate of decrease in area (the \textquotedblleft reduction rate\textquotedblright) of hyper NIR-AF lesions was calculated using the areas of hyper NIR-AF at 6 months (NIRAF\textsubscript{6}) and 48 months (NIRAF\textsubscript{48}):

\[
\text{Reduction rate of hyper NIR - AF lesions} = \frac{\text{NIRAF}_{6}}{\text{NIRAF}_{48}} \\
\]

To compare the NIR-AF images with the RPE-melanin thickness maps at 6 months, retinal vascular patterns of RPE-melanin thickness maps at 6 months were manually aligned with those of NIR-AF images at 6 months, using image processing software (Adobe Photoshop CS5). The magnification of RPE-melanin thickness maps was calibrated using a modified Littman's method.\textsuperscript{29} The square images of RPE-melanin thickness maps with side lengths of 3 mm were used at the corresponding location with the measurement of hyper NIR-AF lesions.

In the acute stage of VKH disease, the presence of serous retinal detachment induces a low-intensity area in AF images,\textsuperscript{10} which impedes the evaluation of RPE change. To avoid the influence of acute exudative inflammation on the imaging analysis, we started evaluations at 6 months after disease onset, following confirmation of complete resolution of any serous retinal detachment during the convalescent/chronic phase of the disease. Thereafter, all subjects were followed until 48 months after onset, and we compared MC-OCT and NIR-AF imaging at 6 and 48 months after onset (Figs. 2–7). For eyes in the chronic/recurrence phase, no recurrences were observed at the time of image acquisition.

Color fundus images covering a 50° visual angle were captured using a Topcon TRC-50 DI retinal camera (Topcon, Tokyo, Japan). Color fundus photographs of each eye were individually evaluated by two blinded observers (T.A. and T.I.) to determine the presence of a sunset glow fundus appearance at 6 and 48 months after onset.

\section*{RESULTS}

Based on subjective evaluation of the color fundus images taken 6 months after onset, 8 eyes from 4 patients were classified as having a sunset glow fundus appearance and 16 eyes from 8 subjects were classified as not having a sunset glow fundus appearance (Table). These classification results coincided with the subjective evaluations at 48 months after onset. The kappa value of interobserver agreement (T.A. and T.I.) was 1.00; that is, the classifications by the two observers agreed for all eyes. Based on our previous PS-OCT study, the presence of sunset glow fundus appearance in VKH disease was assessed as an indicator of the disappearance of choroidal melanin in the DOPU B-scan images.\textsuperscript{16} In DOPU B-scan images, the presence of choroidal melanin was confirmed in all eyes without sunset glow fundus appearance (Figs. 2G, 4D), and disappearance of choroidal melanin was confirmed in all eyes with sunset glow fundus appearance (Figs. 5G, 7D).

Eight eyes of four subjects showed granular hyper-AF lesions in NIR-AF images (Figs. 2B, 5B). The other 16 eyes did not show any particular NIR-AF findings. Among the eyes with NIR-AF lesions, the two eyes of one subject also showed placoid-shaped hyper NIR-AF lesions (Fig. 2B). All eight of the eyes with granular hyper NIR-AF lesions also showed granular hypo NIR-AF lesions (Figs. 2B, 5B). Six of those 8 eyes (75.0%) showed a sunset glow fundus appearance, and 2 of the 16 eyes (12.5%) without NIR-AF lesions showed a sunset glow fundus appearance. The incidence rate of sunset glow fundus appearance was significantly higher in the eyes with NIR-AF lesions than in those without (\(P < 0.0001\); Fisher’s exact probability test).
Multimodal imaging of the right eye of case 1 at 48 months after disease onset. The NIR-AF image (A) shows reduction of the granular hyper-AF lesion (white arrow) and placoid hyper-AF lesion (yellow arrow), and preservation of the granular hypo-AF lesions (red arrow), relative to the appearance at 6 months. The white line designates the scanning line for multicontrast optical coherence tomography in (B–E), and the yellow line designates the scanning line in (G–I). The region enclosed by the white square in the standard OCT B-scan image (B) is shown at high magnification in image (C). Standard OCT B-scan images (B, C) show focal thickening of the retinal pigment epithelium (RPE)-Bruch’s membrane band (C) (white arrow). The degree of polarization uniformity B-scan image (D) shows melanin accumulation at the RPE-Bruch’s membrane band (white arrow) and the presence of choroidal melanin (red arrow). The RPE-melanin cross-sectional image (E) shows RPE-melanin accumulation (white arrow) at the location of hyper NIR-AF lesions. The RPE-melanin thickness map (F) shows a decrease in the area of RPE-melanin accumulation (white arrow) compared with that at 6 months post onset (Fig. 2J). The white square in the standard OCT B-scan image (G) shows the region portrayed in the high-magnification image (H). Standard OCT B-scan images (G, H) demonstrate the recovery from outer retinal damage, with tiny defects at the ellipsoid zone band (white arrows) at the location of the placoid hyper NIR-AF lesion, without particular findings in the RPE-melanin cross-sectional image (I). Scale bar: 500 × 500 μm.
The margins of granular hyper NIR-AF lesions were unclear in SW-AF imaging, and only some parts of the hyper NIR-AF areas showed hyper SW-AF (Figs. 2D, 3, 5D, 6). Hyper NIR-AF lesions were readily observed as high-intensity areas in NIR imaging (Figs. 2C, 3, 5C, 6). In color fundus images, some parts of the granular hyper NIR-AF lesions appeared faintly hyperpigmented (Figs. 2A, 5A). Standard OCT images showed focal thickening of the RPE-Bruch's membrane band at the margins of granular hyper NIR-AF lesions (Figs. 2C, 3, 5C, 6). The RPE Change in VKH Disease.

**FIGURE 5.** Multimodal imaging of the right eye of a 52-year-old male (case 2), with sunset glow fundus appearance, at 6 months after disease onset. Color fundus image (A) shows faint hyperpigmentation in the macula (white arrow). The NIR-AF image (B) shows granular hyper-AF lesions (white arrow) and granular hypo-AF lesions (red arrows). The white line designates the scanning line for multicontrast optical coherence tomography in (E-H). The NIR image (C) illustrates the high reflectivity of granular hyper NIR-AF lesions. The short-wavelength AF image (D) shows indistinct hyper-AF at the location of granular hyper NIR-AF lesions (white arrow). The white square in the standard OCT B-scan image (E) indicates the region shown in the high-magnification image (F). Standard OCT B-scan images (E, F) show focal thickening of the retinal pigment epithelium (RPE)-Bruch's membrane band (white arrow) and hyperpenetration beneath the RPE-Bruch's membrane band (E) (white arrow). The degree of polarization uniformity (DOPU) B-scan image (G) shows the disappearance of choroidal melanin (yellow arrow). The DOPU B-scan image (G) and RPE-melanin cross-sectional image (H) show RPE-melanin accumulation at the granular hyper-AF lesion (white arrow) and focal defects in RPE-melanin at the granular hypo-AF lesion (red arrows). The RPE-melanin thickness map (I) shows RPE-melanin accumulation at granular hyper NIR-AF lesions (white arrow) and decreased RPE-melanin at granular hypo-AF lesions (red arrows). Scale bars: 500 × 500 μm.
location of the granular hyper NIR-AF lesions (Figs. 2F, 4C, 5F, 7C). At the point of the focal thickening, both the external limiting membrane band and the ellipsoid zone band were visible. Both DOPU B-scan images and RPE-melanin cross-sectional images clearly showed melanin accumulations at the RPE-Bruch’s membrane band (Figs. 2G, 2I, 4E, 5G, 5H, 7D, 7E). However, these melanin accumulations were less clear in the chorioretinal melanin thickness maps with PS-OCT, because of the dominance of choroidal melanin (Fig. 2H). In MC-OCT imaging, the RPE-melanin cross-sectional images clearly showed melanin accumulation at the RPE-Bruch’s membrane band (Figs. 2I, 4E, 5H, 7E), without interference from the choroidal melanin, and similarities were found between the granular hyper NIR-AF areas and areas of thickening in RPE-melanin thickness maps (Figs. 2J, 4F, 5I, 7F). This similarity was confirmed at both 12 and 48 months after disease onset in all eyes (Figs. 2J, 4F, 5I, 7F). At 6 months after onset, the area of thickened RPE-melanin (>45 μm) showed a significant positive correlation with the area of hyper NIR-AF lesions ($R^2 = 0.77$, $P = 0.004$, Fig. 8).

In the time-course analysis, granular hyper NIR-AF lesions gradually decreased (Figs. 3, 6). The total areas of granular hyper NIR-AF lesions per eye (mean ± standard deviation [range]) were 0.27 ± 0.20 (0.07–0.53), 0.16 ± 0.15 (0.04–0.44), 0.11 ± 0.09 (0.04–0.29), 0.09 ± 0.09 (0.00–0.529), and 0.05 ± 0.05 (0.00–0.13) mm$^2$ at 6, 12, 24, 36, and 48 months after onset, respectively (Fig. 9). The mean area of granular hyper NIR-AF lesions at 6 months after onset was significantly larger than that at 48 months ($P = 0.012$; Mann-Whitney $U$ test). The reduction rate of hyper NIR-AF lesions did not show a significant correlation with the area of hyper NIR-AF lesions at 6 months ($R^2 = 0.042$, $P = 0.65$, Fig. 10).

Regarding the placoid hyper NIR-AF lesions observed in the first case, SW-AF imaging clearly showed hyper-AF areas (Fig. 2D). Placoid hyper-AF lesions were not clearly visible in the color fundus or NIR images (Figs. 2A, 2C). Standard OCT images showed attenuation of the external limiting membrane band and the ellipsoid zone band, without particular findings at the RPE-Bruch’s membrane band (Fig. 2L). Neither RPE-melanin cross-sectional images nor RPE-melanin thickness maps showed specific findings (Figs. 2J, 2M). The placoid hyper-AF lesions were gradually obscured over time (Fig. 3). At 48 months after onset, standard OCT images showed recovery of the outer retinal layers, with tiny defects at the ellipsoid zone band and a continuous external limiting membrane band (Fig. 4I).

The granular hypo NIR-AF lesions were indistinct in SW-AF, NIR, and color fundus images (Fig. 5D), and only some parts of the hypo NIR-AF lesions showed hypo AF in SW-AF images. Standard OCT images showed hypertransmission beneath the RPE-Bruch’s membrane band (Fig. 5E). Focal defects of RPE-melanin at the hypo NIR-AF lesions were confirmed on DOPU B-scan images, RPE-melanin cross-sectional images, and RPE-melanin thickness maps (Figs. 5G–I). The sizes and shapes of the granular hypo NIR-AF lesions were generally stable throughout the follow-up period (Figs. 3, 6).

**DISCUSSION**

In the present study, we evaluated long-term changes in hyper NIR-AF lesions with concurrent confirmation of RPE-melanin changes with MC-OCT imaging. MC-OCT imaging confirmed the association of RPE-melanin accumulation with granular hyper NIR-AF lesions. In previous studies of chronic VKH disease, a possible association of hyper-AF lesions with focal thickening of RPE-Bruch’s membrane band was reported. However, there were essential limitations in the previous AF studies, due to the lack of topographic information in the AF imaging modalities.
imaging. Furthermore, the lack of RPE-specific contrast in standard OCT imaging impeded the direct comparison between AF and standard OCT. In this study, we used MC-OCT imaging to determine the 3D distribution of RPE-melanin and tried to establish a direct comparison with AF images. As a result, similarities were found between the areas of granular hyper NIR-AF lesions and the thickened areas in RPE-melanin thickness maps. There was a significant positive correlation between the area of granular hyper NIR-AF lesions and the area of thickened RPE-melanin. This MC-OCT finding indicated that granular hyper NIR-AF lesions originate from melanin aggregation at the RPE-Bruch’s membrane band. The presence of hyper SW-AF signals at corresponding locations indicated the simultaneous accumulation of lipofuscin and suggested the presence of RPE aggregation at the RPE-Bruch’s membrane band. Meanwhile, SW-AF signals at hyper NIR-AF lesions were relatively indistinct. One possible reason is blockage of SW-AF signals by macular pigment in the foveal region. However, relatively indistinct hyper SW-AF signals outside the fovea indicated that some of the melanin accumulation occurred without concomitant lipofuscin accumulation. Possible sources for this melanin accumulation are melanin-laden inflammatory cells or extracellular melanin material. The gradual reduction of RPE-melanin accumulation in long-term follow-up showed the reversible nature of this accumulation. Conversely, areas of hypo AF remained unchanged throughout the follow-up period. MC-OCT imaging showed focal RPE damage with melanin loss at hypo NIR-AF lesions and suggested that such focal RPE damage was irreversible.

In histopathological studies of chronic VKH disease, focal proliferation of RPE cells at sites of hyperpigmentation in color fundus images has been reported. Proliferation of RPE cells admixed with inflammatory cells was also reported at depigmented atrophic lesions probably related to resolved Dalén-Fuchs nodules. Based on MC-OCT imaging, we speculated that the granular hyper NIR-AF lesions resulted from RPE hyperplasia or hypertrophy; or from RPE accumulation admixed with melanin-laden inflammatory cells or extracellular melanin material. However, we could not confirm such changes without histopathological findings from the present cases. Moreover, granular hyper NIR-AF lesions in this study showed only subtle hyperpigmentation in color fundus imaging, which differs from the hyperpigmentation or resolving Dalén-Fuchs nodules. Further research is required to confirm the association of granular hyper NIR-AF lesions with hyperpigmentation or resolving Dalén-Fuchs nodules.

**Figure 7.** Multimodal imaging of the right eye of case 2 at 48 months after disease onset. The white line in the NIR-AF image (A) designates the scanning line for multicontrast optical coherence tomography in (B–E). The white square in the standard OCT B-scan image (B) indicates the region shown in the high-magnification image (C). Standard OCT B-scan images (B, C) show focal thickening of the retinal pigment epithelium (RPE)-Bruch’s membrane band (C) (white arrow). The degree of polarization uniformity (DOPU) B-scan image (D) shows the disappearance of choroidal melanin (yellow arrow). The DOPU B-scan image (D) and RPE-melanin cross-sectional image (E) show RPE-melanin accumulation at the site of the granular hyper-AF lesion (white arrows) and a focal defect of RPE-melanin at the granular hypo-AF lesions (red arrows). The RPE-melanin thickness map (F) shows RPE-melanin accumulation at the granular hyper NIR-AF lesions (white arrow) and decreased RPE-melanin at the granular hypo-AF lesions (red arrows). Scale bars: 500 × 500 μm.
This study has established a possible association of granular hyper NIR-AF lesions with the development of a sunset glow fundus appearance. Granular hyper NIR-AF lesions might have developed as a consequence of intense inflammation and be related to the depigmentation of choroidal melanin. NIR-AF imaging could provide useful information regarding treatment strategies for VKH disease. However, NIR-AF imaging is more difficult than NIR or SW-AF imaging, because of the lower NIR-AF intensity. Areas of granular hyper NIR-AF lesions corresponded well with the high-intensity lesions in NIR imaging. NIR imaging could provide significant benefits to clinicians, by revealing melanin accumulation, through widespread usage of NIR imaging in commercial fundus imaging systems.

Placoid hyper NIR-AF lesions in MC-OCT images differed from granular hyper NIR-AF lesions. SW-AF imaging clearly showed hyper-AF signals at placoid lesions. Standard OCT images showed outer retinal damage without the specific findings in RPE-melanin in MC-OCT images. Previous studies with standard OCT have shown outer retinal damage at hyper SW-AF areas in VKH disease. One possible source of the placoid hyper-AF results is a simultaneous increase in both melanin and lipofuscin in RPE cells without aggregation of RPE cells. Increased lipofuscin might induce oxidative damage in the RPE and photoreceptors. At any rate, further study with histopathology is required to confirm the origin of placoid hyper-AF lesions.

This study had several limitations. With the small number of the patients, our study evaluated only some aspects of RPE-melanin change in VKH disease. Further study, with a large number of cases, is required to evaluate in more detail RPE-melanin changes and the relationship between melanin change in the RPE band and choroid. This study did not include histopathological analysis and, therefore, we cannot confirm the presence of melanin accumulation at granular hyper NIR-AF lesions. Measurement of RPE-melanin thickness can be influenced by kernel size of DOPU or melanin packing density in RPE cells. Therefore, RPE-melanin thickness maps do not represent the actual thickness of RPE-melanin, but are only proportional to the thickness of RPE-melanin. Moreover, the interpretation of MC-OCT and PS-OCT findings remains under discussion and there is some controversy about the origin of hyper NIR-AF and hyper SW-AF imaging. Further study is required to answer these important questions.

In conclusion, this study demonstrated the clinical usefulness of MC-OCT imaging for evaluating RPE changes in VKH disease. MC-OCT imaging facilitates 3D evaluation of RPE-melanin change. Multimodal imaging techniques, including MC-OCT, could be applicable in various macular diseases, such as age-related macular degeneration. Furthermore, MC-OCT can simultaneously provide standard OCT and OCT angiography images; hence, MC-OCT has potential to be an all-in-one tool for 3D retinal imaging, and a promising tool for the clinical evaluation of macular diseases.

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