

# Melanin change of retinal pigment epithelium and choroid in the convalescent stage of Vogt-Koyanagi-Harada disease

Ying Huang, Ya-Ting Yang, Bing Lin, Sheng-Hai Huang, Zu-Hua Sun, Rong Zhou, Ying-Zi Li, Xiao-Ling Liu

School of Ophthalmology & Optometry and Eye Hospital, Wenzhou Medical University, Wenzhou 325027, Zhejiang Province, China

**Correspondence to:** Xiao-Ling Liu. School of Ophthalmology & Optometry and Eye Hospital, Wenzhou Medical University, Wenzhou 325027, Zhejiang Province, China. drliuxiaolin@163.com

Received: 2019-12-11 Accepted: 2020-02-18

## Abstract

• **AIM:** To observe the melanin change of the retinal pigment epithelium (RPE) and choroid in the convalescent stage of Vogt-Koyanagi-Harada (VKH).

• **METHODS:** A retrospective study was performed on 40 eyes of 20 patients in the convalescent stage of VKH. Fundus photography (FP), multi-spectral imaging (MSI), and optical coherence tomography (OCT) were performed.

• **RESULTS:** In the VKH convalescent stage, focal RPE melanin accumulation (FRMA) was detected in 34 eyes (85%) on MSI and in 7 eyes (17.5%) on FP. FRMA was limited to the previous retinal detachment area in all 28 eyes (FRMA was detected in 34 eyes on MSI, which were enrolled, and 6 eyes lacked data in the acute stage). Sunset-glow fundus was detected in 20 eyes (50%) on FP. The mean density of FRMA in a 1-mm-diameter circular area of the fovea was  $0.04 \pm 0.07$  on MSI, which was significantly correlated with sunset-glow fundus ( $\rho = 0.467$ ,  $P = 0.02$ ).

• **CONCLUSION:** In the VKH convalescent stage, FRMA is derived from the RPE melanin change, and sunset-glow fundus is derived from the choroid melanin change. A higher density of FRMA in the fovea and sunset-glow fundus represents more serious depigmentation of melanin.

• **KEYWORDS:** melanin; retinal pigment epithelium; choroid; convalescent stage; Vogt-Koyanagi-Harada

**DOI:** 10.18240/ijo.2020.12.13

**Citation:** Huang Y, Yang YT, Lin B, Huang SH, Sun ZH, Zhou R, Li YZ, Liu XL. Melanin change of retinal pigment epithelium and

choroid in the convalescent stage of Vogt-Koyanagi-Harada disease. *Int J Ophthalmol* 2020;13(12):1928-1932

## INTRODUCTION

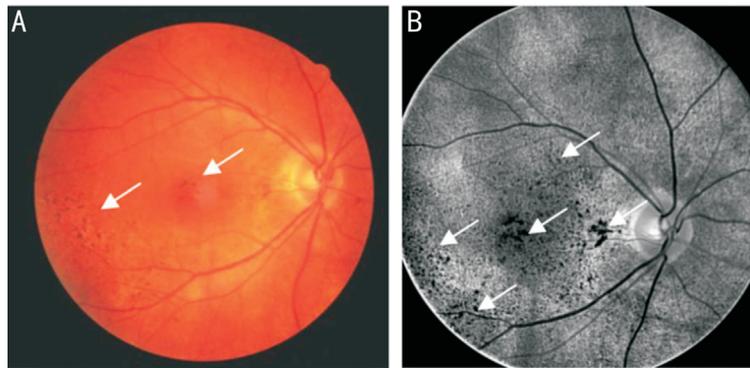
Vogt-Koyanagi-Harada (VKH) disease is a rare systemic inflammatory disease that involves eyes presenting exudative retinal detachment and granulomatous panuveitis. Vogt, Koyanagi and Harada reported the disease independently at the turn of the twentieth century, and Babel called the disease Vogt-Koyanagi-Harada in 1932. Diagnostic criteria were established by the American Uveitis Society in 1978<sup>[1]</sup> and reviewed by an International Committee<sup>[2]</sup>. VKH is a cell-mediated autoimmune disease directed against melanocytes involved in many systems and results in meningeal irritation, hearing impairment, skin depigmentation, and hair whitening or loss, and it tends to affect more pigmented races, such as Asians<sup>[2]</sup>.

The prodromic, uveitic, convalescent and recurrent stages are the four clinical stages of VKH in the eye<sup>[3]</sup>. After four to six weeks, the convalescent stage starts<sup>[4]</sup>. The main clinical features of the convalescent stage include reattachment of the neuro-retina. The melanocytes in the fundus include the retinal pigment epithelium (RPE) and melanophores of the choroid. In this study, we observed the RPE and choroid melanin change in VKH of convalescent stage.

## SUBJECTS AND METHODS

**Ethical Approval** The study was conducted in adherence with the guidelines established by the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice, and it was approved by the Institutional Review Board. Informed consent was obtained from all patients.

This is a retrospective study. All VKH patients were Chinese and naïve, began therapy with systemic prednisone at 1.0 mg/kg·d, which was then followed by slow tapering over 6-12mo. The patient with a duration of VKH was more than 6mo and full reattachment of the neuro-epithelium were eligible for enrolment.



**Figure 1** FRMA of one patient in the convalescent stage of VKH A: The white arrows showed FRMA on FP; B: The white arrows showed FRMA on MSI. Additional melanin was detected by the MSI compared to that detected by the FP.

Forty eyes of 20 patients (9 males and 11 females) with VKH were enrolled. Fundus photography (FP; Canon, CR-1 Mark II), multi-spectral imaging (MSI; RHA, Annidis Corporation, Canada) and optical coherence tomography (OCT; Heidelberg Spectralis OCT) of macular were performed by three professional experienced technicians. Two independent masked experienced retina specialists (Huang Y and Yang YT) evaluated the images. Disagreements between the two specialists were resolved by the third specialist (Liu XL).

Visible abnormal RPE melanin accumulations manifested as black spots on FP. For the MSI, the wavelength of the detecting light was set to ensure the best visibility of the RPE and RPE melanin of Chinese. Normal RPE appeared uniform grey-black background and focal RPE melanin accumulation (FRMA) appeared as black spots on MSI. We use a custom software based on manual threshold method to obtain the density of FRMA in 1 mm diameter circle area of fovea<sup>[5]</sup>. The non-uniform illumination image was corrected by using a technique based on median filtering. The contrast of the corrected image was automated adjusted for manual selection. Then the initial threshold value was obtained as the mean value of image minus 2 times the standard deviation and the center of fovea was manually chosen. All the above methods were implemented using MATLAB (Mathworks, Inc., Natick, MA, USA). The density of FRMA was the area of FRMA in 1 mm diameter circle of fovea/the area of 1 mm diameter circle, and was measured by three times to take the mean value.

Macular OCT was performed. Both the cross line mode (6 mm) and volume mode (25-line consecutive scans, 20°×20°, 5.8×5.8 mm<sup>2</sup>) were scanned to obtain images of the macular. We observe the layer of FRMA on OCT B-scan.

Statistical analysis was performed with SPSS 24.0 (IBM, Chicago, IL) and a 2-sided alpha level of 0.05. Spearman test was using for evaluating the correlation between the density of FRMA in 1 mm diameter circle of fovea and sunset-glow fundus.

## RESULTS

Forty eyes of 20 patients (9 males and 11 females) in the convalescent stage of VKH were enrolled. The mean patient age was 43.80±10.76y. The duration of the disease was 16.10±14.48mo.

In the VKH convalescent stage, RPE melanin accumulated focally in macular defined as FRMA was detected in 34 eyes (85%) on MSI and in 7 eyes (17.5%) on FP. More FRMA was detected on MSI compared with that on the FP of the same eye (Figure 1).

VKH disease is characterized by multifocal retinal detachment at acute stage. We reviewed the earlier data of the 34 eyes whose FRMA were detected on MSI. The retinal detachment typically occurred at acute stage, and FRMA appeared in the retinal detachment area in all 28 eyes (6 eyes were excluded because of a lack of recorded data from the early stage; Figure 2). If the detachment involved the fovea, FRMA was more likely to accumulate in the fovea (Figure 2).

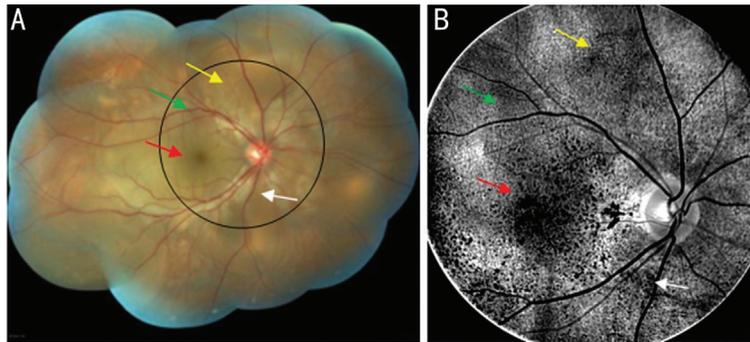
On the macular OCT image, some FRMA was thickening or elevation of the RPE inner layer, whereas some other FRMA was not detected *via* OCT (Figure 3).

Twenty eyes (50%) presented a sunset-glow fundus over a diffuse large area. The sunset-glow area was not consistent with the area of FRMA. 20 eyes (50%) did not present a sunset-glow fundus (Figure 4).

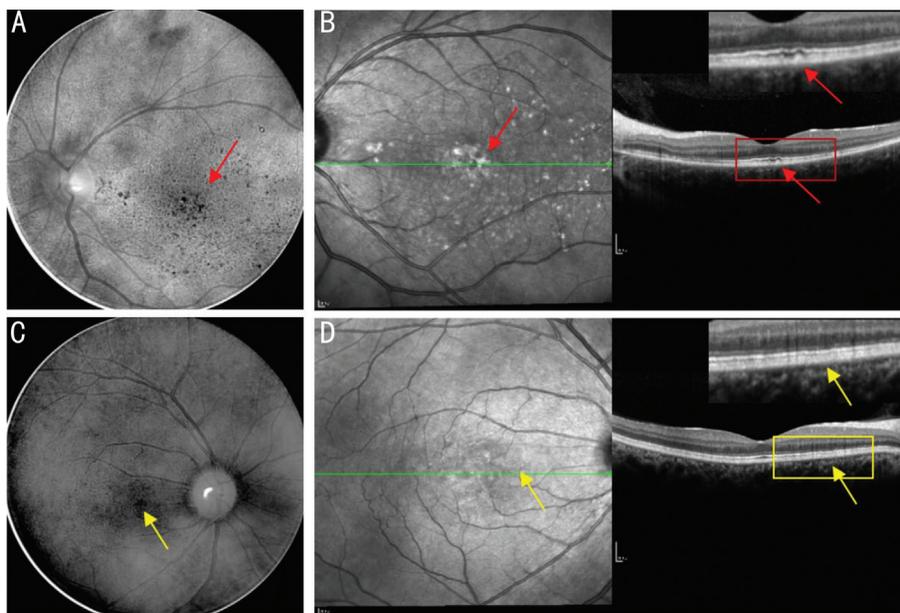
FRMA was detected in 34 eyes on MSI. FRMA was not detected in 1mm diameter circle area of fovea in 9 eyes, and FRMA was detected in 1mm diameter circle area of fovea in the other 25 eyes (Figure 5). The mean density of FRMA of all the 40 eyes in 1 mm diameter circle area of fovea was 0.04±0.07, which was significantly correlated with sunset-glow fundus ( $\rho=0.467$ ,  $P=0.02$ ). The density of FRMA of 6 eyes was more than 0.1, and these 6 eyes were all sunset-glow fundus.

## DISCUSSION

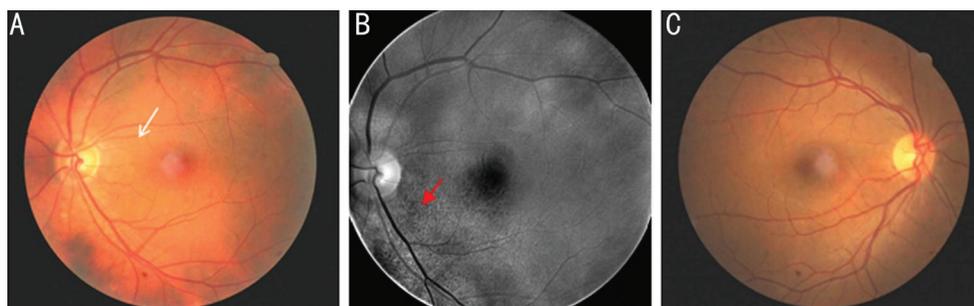
VKH is a cell-mediated autoimmune disease directed against melanocytes<sup>[2]</sup>. RPE cell is a type of melanocyte in the retina, and RPE melanosomes are synthesized in utero and remain



**Figure 2 FRMA appeared in the retinal detachment area of one patient** A: Multifocal retinal detachment was evident in the posterior area on FP of acute stage. The black circle area corresponds to the area of MSI. B: The red arrow shows the detachment involved fovea. FRMA was in the detachment area, and more FRMA was observed in the fovea on MSI of convalescent stage of the same patient. The yellow arrow and white arrow show the detachment uninvolved fovea. No FRMA was in the non-detached healthy area (green arrow).



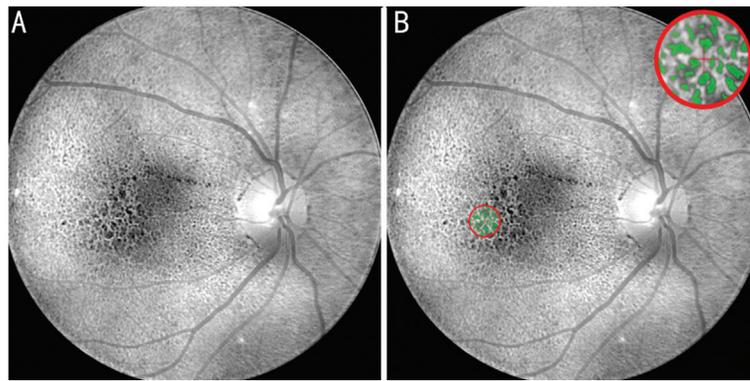
**Figure 3 FRMA on OCT** A: The red arrows showed FRMA on MSI (black spots); B: The OCT image of the same patient of A. The red arrows showed FRMA on OCT IR image, and RPE thickening on OCT tomographic image; C: The yellow arrows showed FRMA on MSI (black spots); D: The OCT image of the same patient of C. OCT IR image and tomographic image were normal.



**Figure 4 Melanin change of choroid** A: Sunset-glow fundus of a diffuse large area (white arrow) on FP; B: MSI of the same patient of A, the area of sunset-glow was not consistent with the area of FRMA on MSI (red arrow); C: FP of another patient showed no sunset-glow fundus.

virtually unchanged thereafter because of the inherent stability of melanin granules<sup>[6]</sup>. When the RPE cell is attacked by VKH and the melanin changed to form FRMA. We speculated that there might be two reasons for FRMA formation. First, cellular necrosis caused by infection, toxins, or trauma results in the

loss of cell membrane integrity and the uncontrolled release of products of cell death into the extracellular space. Melanin in RPE cells is released to be free out of the RPE if the RPE cells die. Second, when the RPE cells are attracted, the melanin in the RPE cells proliferate reactively.



**Figure 5 FRMA on MSI** A: MSI image; B: We choose the center of fovea of A, and the red circle presented the 1 mm diameter circle of fovea, and the green spots presented the FRMA.

In our study, MSI was much more sensitive than FP for detecting RPE and RPE melanin in the VKH convalescent stage, which was consistent with result of prior research<sup>[7]</sup>. Large pigmentary masses can be observed on FP, whereas small masses are difficult to observe because of the limited resolution of FP. Granular hyper lesions were found in 33% patients using near-infrared and autofluorescence (NIR-AF) images of prior research<sup>[8]</sup>. However, in our study, MSI was used for detecting the FRMA, and the rate of detection was 85%, which was much higher. MSI includes a set of en-face sequential retinal images of the posterior pole from the internal limiting membrane (ILM) through the choroid *via* a series of monochromatic discrete light-emitting diodes (LEDs) ranging from 550 to 850 nm in wavelength<sup>[9]</sup>. Individual wavelengths reveal different retinal structures and types of chromophores or absorbing species in the retina and choroid. RPE absorb light of longer wavelengths, like red or infrared light<sup>[9-10]</sup>. MSI is a sensitive non-invasive method to investigate the RPE and RPE melanin<sup>[7]</sup>.

In this study, FRMA was limited in the area of retinal detachment that occurred in the acute period of VKH. RPE has the function of pump, the RPE cells in the area of detachment damaged more seriously. That explain why the area of FRMA was consistent with the area of retinal detachment in the early stage.

Melanin has a higher concentration in the apical aspect than in the basal aspect of the RPE cells<sup>[6]</sup>. On the macular OCT image, some FRMA showed thickening or elevation of the RPE inner layer, which was consistent with Zhou *et al*'s study<sup>[11]</sup>. There was also some melanin accumulated too flatly to be detected by OCT.

Choroidal melanocytes are another cell type that contains melanin, and they are also attacked by the autoimmune system with VKH disease. Other studies have found a variety of choroidal thicknesses during the disease period, with the choroid thickening in the acute period<sup>[12]</sup>, thinning after treatment<sup>[13]</sup>, and equal to or thinner than the normal choroid in the convalescent stage<sup>[12,14-15]</sup>. Diffuse choroidal

inflammation was present during the acute stage generally. Multiple dark foci with loss of choriocapillaris on OCT angiography<sup>[16-17]</sup>, and a reduction in vascular profiles in the choroid on OCT<sup>[18-19]</sup> was observed in VKH patients both in the acute uveitic and convalescent stages. The histopathology of the eyes showed scattering infiltration of lymphocytes in the thickened choroid with a notable disappearance of choroidal melanocytes<sup>[20]</sup>. Sunset-glow fundus was caused by serious choroid depigmentation.

In this study, we measured the FRMA area in the 1 mm diameter circle area of fovea by using a software, and found that the area was significantly correlated with sunset-glow fundus. Miura *et al*<sup>[21]</sup> also found the chronic VKH eyes with granular hyper NIR-AF lesions showed a sunset-glow fundus appearance significantly more frequently than did eyes without such lesions, and the areas of hyper NIR-AF lesions gradually decreased over time. We speculated that higher density of melanin in fovea and sunset-glow fundus presented more serious depigmentation of melanin. Melanin and blood vessel were both black on MSI. The software was unable to distinguish melanin from blood vessel accurately, so we measure the FRMA area only in the 1 mm diameter circle area of fovea where no blood vessels existed.

It's a limitation that normal OCT was performed in this retrospective study, rather than EDI-OCT and OCTA. Choroidal thickness and choroidal vessels were not available in this study. It was regrettable that we could not analyze the relationship between choroidal thickness/choroidal vessels and FRMA.

In this study, we observed the melanin change in VKH convalescent stage. In the future, we will also study the melanin change in VKH acute stage, and try to analyze the melanin change correlation to disease activity.

#### ACKNOWLEDGEMENTS

**Conflicts of Interest:** Huang Y, None; Yang YT, None; Lin B, None; Huang SH, None; Sun ZH, None; Zhou R, None; Li YZ, None; Liu XL, None.

**REFERENCES**

- 1 Snyder DA, Tessler HH. Vogt-Koyanagi-Harada syndrome. *Am J Ophthalmol* 1980;90(1):69-75.
- 2 O'Keefe GAD, Rao NA. Vogt-Koyanagi-Harada disease. *Surv Ophthalmol* 2017;62(1):1-25.
- 3 Sugiura S. Vogt-Koyanagi-Harada disease. *Jpn J Ophthalmol* 1978; 22:9-35.
- 4 Arellanes-García L, Hernández-Barrios M, Fromow-Guerra J, Cervantes-Fanning P. Fluorescein fundus angiographic findings in Vogt-Koyanagi-Harada syndrome. *Int Ophthalmol* 2007;27(2-3): 155-161.
- 5 MacGillivray TJ, Patton N, Doubal FN, Graham C, Wardlaw JM. Fractal analysis of the retinal vascular network in fundus images. *Annu Int Conf IEEE Eng Med Biol Soc* 2007;2007:6456-6459.
- 6 Weiter JJ, Delori FC, Wing GL, Fitch KA. Retinal pigment epithelial lipofuscin and melanin and choroidal melanin in human eyes. *Invest Ophthalmol Vis Sci* 1986;27:145-152.
- 7 Huang G, Peng JC, Ye Z, Kijlstra A, Zhang DL, Yang PZ. Multispectral image analysis in Vogt-Koyanagi-Harada disease. *Acta Ophthalmol* 2018;96(4):411-419.
- 8 Miura M, Makita S, Azuma S, Yasuno Y, Sugiyama S, Mino T, Yamaguchi T, Agawa T, Iwasaki T, Usui Y, Rao NA, Goto H. Evaluation of retinal pigment epithelium layer change in Vogt-Koyanagi-Harada disease with multicontrast optical coherence tomography. *Invest Ophthalmol Vis Sci* 2019;60(10):3352.
- 9 Everdell NL, Styles IB, Calcagni A, Gibson J, Hebden J, Claridge E. Multispectral imaging of the ocular fundus using light emitting diode illumination. *Rev Sci Instrum* 2010;81(9):093706.
- 10 Styles IB, Calcagni A, Claridge E, Orihuela-Espina F, Gibson JM. Quantitative analysis of multi-spectral fundus images. *Med Image Anal* 2006;10(4):578-597.
- 11 Zhou M, Jiang CH, Gu RP, Sun ZC, Huynh N, Chang Q. Correlation between retinal changes and visual function in late-stage Vogt-Koyanagi-Harada disease: an optical coherence tomography study. *J Ophthalmol* 2015;2015:916485.
- 12 Fong AH, Li KK, Wong D. Choroidal evaluation using enhanced depth imaging spectral-domain optical coherence tomography in Vogt-Koyanagi-Harada disease. *Retina* 2011;31(3):502-509.
- 13 Agrawal R, Li LKH, Nakhate V, Khandelwal N, Mahendradas P. Choroidal vascularity index in vogt-koyanagi-harada disease: an EDI-OCT derived tool for monitoring disease progression. *Trans Vis Sci Tech* 2016;5(4):7.
- 14 Nazari H, Hariri A, Hu ZH, Ouyang YW, Sadda S, Rao NA. Choroidal atrophy and loss of choriocapillaris in convalescent stage of Vogt-Koyanagi-Harada disease: *in vivo* documentation. *J Ophthalmic Inflamm Infect* 2014;4:9.
- 15 Takahashi H, Takase H, Ishizuka A, Miyanaga M, Kawaguchi T, Ohno-Matsui K, Mochizuki M. Choroidal thickness in convalescent Vogt-Koyanagi-Harada disease. *Retina* 2014;34(4):775-780.
- 16 Aggarwal K, Agarwal A, Mahajan S, Invernizzi A, Mandadi SKR, Singh R, Bansal R, Dogra MR, Gupta V. The role of optical coherence tomography angiography in the diagnosis and management of acute Vogt-Koyanagi-Harada disease. *Ocular Immunol Inflamm* 2018;26(1):142-153.
- 17 Cennamo G, Romano MR, Iovino C, de Crecchio G, Cennamo G. Optical coherence tomography angiography in incomplete acute Vogt-Koyanagi-Harada disease. *Int J Ophthalmol* 2017;10(4):661-662.
- 18 Li M, Liu QH, Luo Y, Li YH, Lin SF, Lian P, Yang QF, Li XF, Liu XL, Sadda S, Lu L. Enhanced depth SD-OCT images reveal characteristic choroidal changes in patients with Vogt-Koyanagi-Harada disease. *Ophthalmic Surg Lasers Imaging Retina* 2016;47(11):1004-1012.
- 19 Invernizzi A, Agarwal A, Cozzi M, Viola F, Nguyen QD, Staurengi G. Enhanced depth imaging optical coherence tomography features in areas of choriocapillaris hypoperfusion. *Retina* 2016; 36(10):2013-2021.
- 20 Inomata H, Sakamoto T. Immunohistochemical studies of Vogt-Koyanagi-Harada disease with sunset sky fundus. *Curr Eye Res* 1990;9(sup1):35-40.
- 21 Miura M, Makita S, Azuma S, *et al.* Evaluation of retinal pigment epithelium layer change in Vogt-Koyanagi-Harada disease with multicontrast optical coherence tomography. *Invest Ophthalmol Vis Sci* 2019;60(10):3352.