Evidence of Macular Pigment in the Central Macula in Albinism

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2 Abstract

3 **Purpose:** Albinism represents a spectrum of disorders with diminished to absent
4 amounts of melanin pigmentation including the posterior segment of the eye. Macular
5 pigment (MP) consists of two main carotenoids, lutein and zeaxanthin, concentrated in
6 the macula. MP serves as blue light absorbent, antioxidant, and may reduce chromatic
7 aberration and glare. It remains unclear if albinos have detectable MP. The purpose was
8 to investigate the distribution of MP in albino patients with psychophysical and imaging
9 techniques.

10 **Methods:** MP was measured at the eccentricity of 0.5 degrees by heterochromatic
11 flicker perimetry (QuantifEye®; Tinsley Precision Instruments Ltd.) or by scanning laser
12 ophthalmoscopy (MPOD module, MultiColor Spectralis®, Heidelberg Engineering,
13 Heidelberg, Germany) in four enrolled albino patients, who were also investigated with
14 multimodal ophthalmic imaging.

15 **Results:** Visual acuity ranged from 20/32 to 20/125, nystagmus was present in three
16 patients, and all patients showed typical foveal hypoplasia on fundus exam and optical
17 coherence tomography. Fundus autofluorescence (FAF) demonstrated various degrees
18 of central FAF signal attenuation. Genetic testing was available in three patients and
19 confirmed the diagnosis. Measurable amounts of MP were detected in all four patients
20 and ranged from 0.05 to 0.24, which is below the normal expected range.

21 **Conclusions:** We conclude that MP can be demonstrated and measured in albinos.
22 Further studies are needed to investigate MP accumulation following carotenoid
23 supplementation.

24 **Key words:** albinism, macular pigment, foveal hypoplasia, lutein, zeaxanthin, diet

25 supplementation

26

Introduction

Ocular and oculocutaneous albinism represent a spectrum of disorders with absent or significantly diminished amount of melanin either across different body tissues (OCA1 and OCA2), or exclusively in eye tissues (OA1). (Summers, 2009) Clinical findings and visual performance show significant variability without obvious phenotype-genotype correlation. (Gargiulo et al., 2011) Typically, the ocular phenotype includes iris transillumination, foveal hypoplasia, nystagmus, reduced best-corrected visual acuity, refractive errors, photosensitivity, and abnormal development of the visual pathways with characteristic abnormal routing of ganglion cell axons in the chiasma, resulting in abnormal pattern visually evoked potentials. (Dorey et al., 2003) Current treatment options are limited to low vision aids. Previously correlation was found between the amount of melanin fundus pigmentation and visual function in albino patients. (Summers, 1996)

The macular pigment (MP) consists of two main carotenoids, lutein and zeaxanthin, which are concentrated in the macula. (Whitehead et al., 2006) MP is hypothesized to function as a protector by absorbing blue light incident on the retina, thereby reducing photo-oxidative stress to the underlying photoreceptors. It is also thought to improve visual function via reduction of chromatic aberration and glare. (Wooten and Hammond, 2002) The only report on macular pigment in albino patients failed to demonstrate any MP. (Abadi and Cox, 1992) The MP would be a hypothetical candidate to improve visual performance, simply by increasing pigmentation, reducing light scatter and thus glare sensitivity. MPOD normal range in US population has been estimated to be 0.24-0.45. Even in normal subjects, supplementation and an increase in the macular pigment optical density (MPOD) could be correlated with increased visual performance in randomized, placebo-controlled studies. (Nolan et al., 2011, Trieschmann et al., 2007,

van den Berg et al., 2009) As this pigment is not produced in the retina, but is absorbed via diet, it can be manipulated by diet and supplementation and thereby providing an opportunity to positively impact visual performance. It needs to be determined if MP is detectable at all in albinism.

In this case series, we evaluated 4 albino patients and performed measurements of MP: in three patients by heterochromatic flicker photometry (HFP), and in one patient with the novel MPOD module of the Heidelberg MultiColor Spectralis®.

Methods

Four albino patients were enrolled. Multimodal retinal imaging was performed. MPOD measurements were performed with the QuantifEye® device in 3 albino patients, and an additional patient underwent MPOD evaluation with the new MPOD module of the Heidelberg Multicolor Spectralis®. The study followed the tenets of the Declaration of Helsinki and was approved by the local Ethics Review Board.

The most widely used technique to assess the MPOD is the HFP method: A small (usually about 1 degree) visual stimulus with alternating blue (wavelength 465 nm, maximally absorbed by MP) and green (wavelength 530 nm, not significantly absorbed by MP) light is presented. We used the QuantifEye® device (QuantifEye®; MPS 9000 series: Tinsley Precision Instruments Ltd., Croydon, Essex, UK) for HFP. The device was first described in 2009 (van der Veen et al., 2009a), and showed good repeatability ($r=0.97$), and produced similar results to retinal reflectometry (van der Veen et al., 2009b) and MultiColor Spectralis® HRA (Dennison et al., 2013). The device provides the option to either test only the central MPOD (0.5 degrees eccentricity) or perform both the central and the peripheral test of MPOD (performed at 6 degrees eccentricity). In resemblance to the visual field test, during MPOD testing the patient is required to press

the button, once he notices the appearance of the flicker at the stimulus spot. While performing central measurement, the flicker stimulus is presented centrally at the fixation point, and for peripheral measurement, the flicker is presented at the same central location, however the fixation point is moved to the periphery of the presented field. Peripheral measurement can be challenging due to the required peripheral fixation. If the peripheral measurement is not performed for any reason, the central measurement is estimated based on age-adjusted data, taking into account the “normal yellowing” of the natural human lens. We attempted both, central and peripheral testing in our patients. The measurements were performed with non-dilated pupils, in dim light settings and with habitual correction, based on manufacturer’s instructions.

The MPOD module of Spectralis® uses the MultiColor Spectralis® platform instrument as the basis. In difference to the BluePeak system used to acquire blue AF images on the (standard) Heidelberg Spectralis®, it is equipped with an additional green laser, resulting in the ability to acquire infrared, green, and blue reflectance images from the retina simultaneously. For the MP measurements, the fluorescence detection is used to measure MP. The built-in software module performs the final MPOD calculation (as described by Trieschmann et al.(Trieschmann et al., 2006)) Once the measurement is performed, the average MPOD can be calculated at any eccentricity covered by the original scan (5 degrees) by the in-built software. In our study we report 0.5 eccentricity measurements, similar to the eccentricity used by the QuantifEye® device. The measurements were performed with dilated pupils, in dim light settings, and with optimal visualization of the anatomical features on the preceding red-free scan, utilizing optimization of focal distance and magnification.

Results:

The clinical characteristics of the 4 albino patients are summarized below (only positive and disease-specific findings are noted in the descriptions below, while normal findings are omitted):

Patient 1: forty two-year-old male with sporadic oculocutaneous albinism. His best-corrected visual acuity was 20/63 in the right eye and 20/50 in the left; no nystagmus but mild iris transillumination were noted on ocular exam. On dilated fundus examination a low-pigmented fundus and absence of the foveal reflex were noted. Foveal hypoplasia was confirmed by OCT (Figure 1, B1). Molecular diagnosis is not available for this patient. The macular pigment measured by QuantifEye was 0.05 in the right eye, with reliable central and peripheral measurements, and not measurable in the left eye due to unreliable central and peripheral measurements.

Patient 2: Fifty-year-old male with oculocutaneous albinism. His best-corrected visual acuity on exam was 20/63 in the right and 20/50 in the left eye with hyperopic astigmatic correction. He had pendular nystagmus, moderate iris transillumination and lack of the foveal reflex. OCT scan confirmed foveal hypoplasia in both eyes (Figure 1, B2). Genetic testing was consistent with OCA1 (*TYR* gene *Compound Heterozygous Mutation: c.61C>T (p.Pro21Ser) in exon 1, c.1037-7T>A (IVS2-7T>A) in exon 3*). The macular pigment measured by QuantifEye was 0.14 in the right eye, with reliable central but unreliable peripheral measurements, and not measurable in the left eye due to significant nystagmus.

Patient 3: sixty five-year-old male, with best-corrected visual acuity of 20/80 in the right and 20/50 in the left eye, pendular nystagmus, absence of iris transillumination and foveal hypoplasia in both eyes as detected by dilated fundus examination and confirmed

by OCT (Figure 1, B3). Additional clinical findings included bilateral hypermetropia, alternating exotropia, and nuclear sclerosis of the lens in the right eye. He had a positive family history of albinism: two affected siblings, daughter and granddaughter. He reported no history of consanguinity in the family. Genetic testing was consistent with OCA2 (*Single Heterozygous Mutation: c.1103C>T (p.Ala368Val) in exon 10*). Macular pigment measured by QuantifEye was 0.12 in the right eye, with reliable central and peripheral measurements, and 0.24 in the left eye with reliable central and unreliable peripheral measurements.

Patient 4: forty-year-old male with oculocutaneous albinism. He was emmetropic, and his uncorrected visual acuity was 20/40 in the right eye and 20/32 in the left. There was no nystagmus and no iris transillumination on ocular exam. Foveal hypoplasia was detected in both eyes on dilated fundus exam, and confirmed by OCT (Figure 1, B4). Homozygous p.Val443Ile:c.1327G>A mutation was detected, consistent with the diagnosis of OCA2. Macular pigment measured by MultiColor was 0.15 in the right eye, and 0.18 in the left eye. (Figure 2)

Figure 1 shows retinal images of the right eye of each patient. As can be observed on fundus autofluorescence imaging (Figure 1, C1-C4), different degrees of foveal AF signal attenuation due to absorbance by the MP can be detected: while in patients 1 and 2, there appears to be only a weak, but definite central attenuation of the AF signal, patient 4, and even more so, patient 3 demonstrated quite normal distribution of AF signal with central AF attenuation. OCT scans of all 4 patients demonstrated foveal hypoplasia with complete absence of the foveal pit and presence of all inner retinal layers throughout the fovea (Figure 1, B1-B4).

MP measurements with the QuantifEye® were technically possible in 5 out of 6 eyes of

Patients 1, 2 and 3. Central MPOD measurements were possible in all 5 eyes, however appeared unreliable (based on a QuantifEye® software output) in 1 out of 5 eyes (left eye of Patient 1). The peripheral MPOD measurements were technically possible in only 2 out of 4 eyes (right eye of Patient 1 and right eye of Patient 3). In Patient 3, MPOD in the right eye was 0.12, as calculated from central and peripheral measurements. In the left eye, the peripheral measurement of MPOD appeared to be unreliable, and therefore the final MPOD was based on age-matched data, and was estimated to be 0.24. We believe that the inter-eye discrepancy of MPOD in Patient 3 can be explained by presence of nuclear cataract in this patient. In Patient 4, the MPOD measurement was performed by means of the MultiColor Spectralis®, and measured 0.15 in the right eye and 0.18 in the left eye. The differential map of blue and green fundus autofluorescence clearly shows accumulation of MP in the central retina (Figure 2).

Discussion

The existence and distribution of MP in albino patients has not been previously evaluated by currently available techniques: one single report from 1992 concluded that MPOD is not measurable. In one recent report of MPOD in an oculocutaneous albinism patient, the value of MPOD was found to be 0.1, as measured by HFP technique.(Putnam and Bland, 2014) The Macular Pigment Reflectometer (MPR) (van de Kraats et al., 2006) device would offer an “objective” method; however there are limitations to use the device in certain conditions (such as albinism) due to its low reliability in performing measurements in absence of choroidal and retinal melanin. The MPOD measurement with the novel MPOD module of the MultiColor Spectralis® provides objective measurements of the macular pigment. In our patient, a low but measurable amount of MP was detected. The spatial MP profile showed an elevated

central plateau as opposed to a more pronounced central or pericentral peak that is usually demonstrated in normal retinas. It must be noted that the shortcomings of the HFP psychophysical technique for MP measurement are possibly further aggravated by the impaired central visual acuity, nystagmus and poor fixation in albino patients, and can lead to substantial MPOD measurement artifacts in albino population. Our data suggest that absence of the normal foveal pit development does not preclude MP deposition and that the fovea is still being “recognized”, at least to some degree, by MP molecule transport mechanisms. We detected low levels of MP in four albino patients; however clinical significance of such low levels regarding their impact on visual performance remains unclear. The forthcoming LUVIA (LUtein for Vision in Albinism) clinical trial will investigate MP accumulation following carotenoids supplementation and its consequences for visual performance in albino patients (ClinicalTrials.gov Identifier: NCT02200263).

In conclusion, we demonstrate measurable (although low) levels of MP in albino patients, ranging from 0.05 to 0.24. Further studies aiming to provide more cross-sectional data on MPOD distribution as well as a hypothesized increase in MPOD following carotenoid supplementation in this population may be warranted.

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Figure 1: Red free, optical coherence tomography (OCT) and fundus autofluorescence (FAF) images (Spectralis®, Heidelberg Engineering).
Left column (A1-A4) represents red free reflectance images of Patient 1-4 correspondingly.
Middle column (B1-B4) represents horizontal cross-sectional high resolution foveal OCT B-scans of Patients 1-4 correspondingly showing foveal hypoplasia in all 4 patients.
Right column (C1-C4) shows FAF scans. The FAF images (488nm, HRA2, Heidelberg Engineering; acquired with 100% laser power and 90/91% sensitivity settings) show topographic distribution of autofluorescent (AF) signal in the right eye of Patients 1-4, correspondingly. Attenuation of AF signal in the foveal center, which is usually attributed to presence of macular pigment, can be observed in all patients, however to a different degree. (Symmetrical findings were observed in the contralateral eye of each patient).
MPOD values were 0.05, 0.14, and 0.12 for Patients 1-3, correspondingly, as measured by QuantifEye® device; and 0.15 for Patient 4, as measured by MPOD module of Multicolor Spectralis®.

Figure 2: MPOD measurement by MPOD module of MultiColor Spectralis®, Heidelberg Engineering.
Blue (488 nm) and green (518 nm) autofluorescence image is acquired simultaneously by dual-wavelength cSLO system of the device (A, C). Differential topographic map of AF is calculated by in-built software (Heidelberg Eye Explorer (HEYEX)), and radial density map of MP distribution is created (B, D). The red and purple markers can be moved to provide the relevant averaged MPOD reading for the area of interest. In our patient 0.5 degrees average MPOD (as pointed by the red marker) was 0.15 in the right eye and 0.18 in the left eye. (The 0.5 degrees area of MPOD measurement corresponds to the MPOD measurement area evaluated by QuantifEye® device)

Figure 1

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288 Figure 2