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Arrested development: High-resolution imaging of foveal morphology in albinism

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ABSTRACT

Albinism, an inherited disorder of melanin biosynthesis, disrupts normal retinal development, with foveal hypoplasia as one of the more commonly associated ocular phenotypes. However the cellular integrity of the fovea in albinism is not well understood – there likely exist important anatomical differences that underlie phenotypic variability within the disease and that also may affect responsiveness to therapeutic intervention. Here, using spectral-domain optical coherence tomography (SD-OCT) and adaptive optics (AO) retinal imaging, we obtained high-resolution images of the foveal region in six individuals with albinism. We provide a quantitative analysis of cone density and outer segment elongation demonstrating that foveal cone specialization is variable in albinism. In addition, our data reveal a continuum of foveal pit morphology, roughly aligning with schematics of normal foveal development based on post-mortem analyses. Different albinism subtypes, genetic mutations, and constitutional pigment background likely play a role in determining the degree of foveal maturation.

1. Introduction

Albinism is an inherited disorder of melanin biosynthesis, associated with absent or reduced melanin pigment in the eye, and often in the skin and hair. Oculocutaneous albinism type 1 (OCA1; MIM 203100) is a recessive disorder in which individuals have a mutation in the tyrosinase gene on chromosome 11q14.3. Those who never develop any melanin pigment within the eye, hair, and skin have OCA1A, while those with development of some melanin pigment due to a "leaky" mutation that allows residual enzyme activity have OCA1B (King et al., 2003). Ocular albinism (OA1; MIM 300500) is characterized by X-linked inheritance and has been mapped to Xp22.3–Xp22.2. The affected males typically have normal skin and hair pigment, but will usually have all the ocular-visual manifestations of albinism, including iris transillumination, macular translucency, photosensitivity, refractive errors, astigmatism, nystagmus, impaired stereopsis, altered retinostriate decussation, and reduced visual acuity (Oetting, Summers, & King, 1994; Summers, 2009).

The normal human fovea underlies the majority of our visual function, including color vision and high spatial acuity vision, and is characterized by an avascular zone, an increase in cone photoreceptor density, and an excavation of inner retinal neurons (Hendrickson, 2005). Foveal manifestations of albinism can include absence of a foveal avascular zone (Abadi & Pascal, 1989; Gregor, 1978), foveal hypoplasia (absence of a foveal pit) (Kinnear, Jay, & Witkop, 1985; Oetting et al., 1994), and loss of an annular reflex (Lee, King, & Summers, 2001). Reductions in visual acuity have been attributed to absent melanin synthesis and the absence of a foveal pit (Seo et al., 2007), however certain patients with some melanin pigment in their maculae due to residual enzyme activity still show decreased visual acuity and others with poorly defined foveal pits have relatively preserved visual acuity (Harvey, King, & Summers, 2006; Summers, 1996). As such, significant work remains in elucidating the relationship between foveal maldevelopment and visual function in albinism (Jeffery, 1997). This lack of clarity stems in part from ambiguity surrounding foveal anatomy in albinism, specifically with regard to foveal cone specialization.

There are but a few cases in the literature that deal directly with the issue of cone specialization in human albinism, and they are far from unified in their findings. In a post-mortem analysis, Fulton, Albert, and Craft (1978) found that central cone density in a patient

Abbreviations: AO, adaptive optics; ELM, external limiting membrane; IS, inner segment; ILM, internal limiting membrane; OA, ocular albinism; OCA, oculocutaneous albinism; OCT, optical coherence tomography; ONL, outer nuclear layer; OPL, outer plexiform layer; OS, outer segment; RPE, retinal pigment epithelium.

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with OCA was significantly *decreased* compared to values normally found in the parafoveal region (Fulton et al., 1978). In another histological study on an OCA retina, *increased* cone density was observed in the fovea compared to the periphery, though it was not reported whether the foveal density was consistent with normal values (Akeo et al., 1996). Wilson, Mets, Nagy, and Kressel (1988) measured grating acuity in two OCA patients and found a defect in spatial processing in the central retina that was best explained by an increased spacing of the central cone photoreceptors. Based on multifocal electroretinogram findings, it has been inferred that the density of cone photoreceptors across the central retina is homogeneous in OCA (Kelly & Weiss, 2006) and in ocular albinism (OA) (Nusinowitz & Sarraf, 2008), consistent with an underdeveloped macular region.

Recent advances in high-resolution imaging make it possible to quantitatively assess foveal morphology in the living retina. Optical coherence tomography (OCT) offers high axial resolution and enables visualization of retinal lamination, while adaptive optics (AO) provides high lateral resolution and allows direct visualization of the cone photoreceptor mosaic. Here we applied these imaging tools to characterize the foveal cone specialization in four individuals with OCA1B and two with OA1. We observed varying degrees of foveal maturity and cone specialization in the albinotic retina, which roughly align with schematics of normal foveal development based on post-mortem analyses (Isenberg, 1986; Mann, 1950; Provis, Diaz, & Dreher, 1998; Springer, 1999). This supports the idea that, in general, normal foveal development is arrested in individuals with albinism (Wilson et al., 1988). As such, examining foveal morphology and cone specialization in albinism may offer valuable insight into the process of normal foveal development. In addition, as previous work suggests that it may be possible to recover some aspect of foveal maturity in albinism through L-DOPA supplementation (Ilia & Jeffery, 1999, 2000; Lopez, Decatur, Stamer, Lynch, & McKay, 2008; Reis, Ventura, Kubrusly, de Mello, & de Mello, 2007), better, more quantitative measures of foveal architecture in albinism are needed. The tools described here should prove useful for dissecting retinal versus cortical roles in vision loss in albinism, evaluating therapeutic strategies for albinism, and for more accurately characterizing post-natal foveal development in the normal retina.

2. Methods

2.1. Human subjects

This study adhered to the tenets of the Declaration of Helsinki and was approved by the Children's Hospital of Wisconsin Institutional Review Board. Participants provided written informed consent after explanation of the nature and possible consequences of the study. Two subjects with X-linked OA1 (both male), four with OCA1B (three male and one female), and 167 control subjects without albinism (72 males, 95 females with a mean age of 32.6) were recruited for retinal imaging. The control subjects excluded individuals with color vision deficiency or history of ocular surgery or other diagnosed retinal abnormalities. Fundus photographs were obtained from the subjects with albinism and were evaluated for fundus pigmentation, foveal reflex, foveal vasculature, and general anatomical features. These photos, in addition to clinical examination, were used to classify each of the subjects with albinism by phenotype. A summary of clinical characteristics can be found in Table 1.

2.2. Spectral-domain optical coherence tomography (SD-OCT)

Volumetric images of the macula were acquired using the Zeiss Cirrus HD-OCT (Carl Zeiss, Meditec, Dublin CA, USA). Volumes were 6 mm × 6 mm and consisted of 128 B-scans (512 A-scans/B-scan). At least three replicate scans were acquired to assess repeatability of fixation and allow an estimation of the preferred retinal locus of fixation. For each subject with albinism, images containing a Cirrus LSO (laser scanning ophthalmoscope) map, retinal thickness map, and volume scan boundaries were extracted and registered to a color fundus image using i2k Align Retina software (DualAlign, LLC, Clifton Park, NY USA). The LSO images with OCT boundaries contain a cross hair depicting the center of the scan, which by design, corresponds to fixation for that particular volume scan. After all replicate Cirrus LSO maps were registered to the color fundus image, the center of fixation was identified for each image, and their absolute positions within the fundus recorded. The center of mass of these locations was calculated to derive a single estimate of the preferred retinal locus of fixation. In addition, the position of the center of the retinal doming was approximated, and its position within the fundus recorded for each subject. The expected location of the fovea was also calculated based on data from Rohrschneider (2004). Some subjects had cyclotropia, and their photos were corrected prior to calculation of the expected foveal location (Bixenman & Von Noorden, 1982). In all cases, the locus of fixation was close to the expected foveal location, however errors in this calculation could affect our absolute topographical analysis of cone density.

High-resolution SD-OCT (Bioptigen, Inc., Durham, NC) imaging was performed in all six subjects with albinism and the 167 controls. Line scan sets were acquired (1000 A-scans/B-scan; 100 repeated B-scans) through the center of the foveal center (either the actual fovea or expected foveal location when there was no visible pit). Scans were registered and averaged as previously described to reduce speckle noise in the image (Tanna et al., 2009).

The inner limiting membrane (*ILM*), external limiting membrane (*ELM*), inner segment-outer segment junction (*IS/OS*), and two layers representing the retinal pigment epithelium (*RPE1, RPE2*) were manually segmented. Demarcated retinal layers were then interpolated and layer thicknesses were extracted using programs developed with Matlab™ (Mathworks, Natick, MA). The distance between the ILM and RPE2 provided total retinal thickness, the distance between the center of the ELM and center of the IS/OS junction provided inner segment (IS) thickness, while the distance between the center of the IS/OS and center of RPE1 provided the outer segment layer (OS) thickness. The scan length of each OCT scan set was corrected for inter-individual differences in axial length based on Bennett, Rudnicka, and Edgar (1994). Interpolated thickness values for each subjects' IS and OS layer were binned and averaged at 0.1 mm incre-

Table 1
Clinical summary of subjects with albinism.

Subject	Sex	Age	Diagnosis	Foveal pit (on OCT)	Visual acuity (BCVA)	Axial length (OD)	Axial length (OS)	Nystagmus
JC103	M	16	OA1	None	20/70	22.58	22.37	Horizontal pendular
JC0125	M	27	OCA1B	None	20/60+	22.49	22.08	Horizontal pendular
AD0063	M	32	OA1	None	20/40	24.16	26.97	None
JC0140	M	10	OCA1B	Indistinct	20/20−3	22.06	22.08	None
JC0170	F	16	OCA1B	Indistinct	20/40	23.84	23.62	Horizontal pendular
JC0131	M	20	OCA1B	Indistinct	20/40+	24.94	24.87	Horizontal pendular