Published in final edited form as:

Adv Exp Med Biol. 2016 ; 854: 285–290. doi:10.1007/978-3-319-17121-0_38.

Quantitative Fundus Autofluorescence in Best Vitelliform Macular Dystrophy: RPE Lipofuscin is not Increased in Non-Lesion Areas of Retina

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Abstract

Since the lipofuscin of retinal pigment epithelial (RPE) cells has been implicated in the pathogenesis of Best vitelliform macular dystrophy, we quantified fundus autofluorescence (quantitative fundus autofluorescence, qAF) as an indirect measure of RPE lipofuscin levels. Mean non-lesion qAF was found to be within normal limits for age. By spectral domain optical coherence tomography (SD-OCT) vitelliform lesions presented as fluid-filled subretinal detachments containing reflective material. We discuss photoreceptor outer segment debris as the source of the intense fluorescence of these lesions and loss of anion channel functioning as an explanation for the bullous photoreceptor-RPE detachment. Unexplained is the propensity of the disease for central retina.

Keywords

Best vitelliform macular dystrophy; *BEST1*; Fundus autofluorescence; Quantitative autofluorescence; SD-OCT

38.1 Introduction

The inherent fluorescence of retina originates primarily from the lipofuscin of retinal pigment epithelial (RPE) cells and is commonly imaged as fundus autofluorescence (AF) by confocal laser scanning ophthalmoscopy (cSLO). The lipofuscin fluorophores that have been described are vitamin A aldehyde adducts with excitation maxima from ~ 430–510 nm and peak emission of ~ 600 nm. Topographic patterns of fundus AF are well known to be altered in age-related macular degeneration, retinitis pigmentosa, acute macular disease, pattern dystrophies and Bull's eye maculopathy (von Ruckmann et al. 1997b; Robson et al. 2006; Boon et al. 2007; Kellner et al. 2009; Michaelides et al. 2010; Gelman et al. 2012). Fundus autofluorescence intensity is particularly elevated in recessive Stargardt disease (STGD1) (Delori et al. 1995a; Lois et al. 2004; Cideciyan et al. 2005). Emission spectra recorded at the fundus in healthy eyes and in patients with STGD1 and age-related macular degeneration all exhibit emission maxima at 580–620 nm (Delori et al. 1995b; Delori et al. 1995a).

38.2 Best Vitelliform Macular Dystrophy: Clinical Findings

Best vitelliform macular dystrophy (BVMD) is an autosomal dominant disease associated with mutations in *BEST1*, the gene encoding the bestrophin-1 protein located on the basolateral membrane and within intracellular compartments of RPE cells (Petrukhin et al. 1998; Marmorstein et al. 2000). Ophthalmoscopic features of BVMD typically present in juveniles, and overt disease is most often limited to the macula (Boon et al. 2009). Aberrant responses recorded by electrooculography (EOG) can be diagnostic (Deutman 1969). The onset of the disorder is usually characterized by a central oval lesion (vitelliform lesion) that exhibits intense fluorescence in fundus AF images (Spaide et al. 2006) (Fig. 38.1a) and that is visible as a dome shaped separation between photoreceptor cells and RPE in images acquired by spectral domain optical coherence tomography (SD-OCT) (Querques et al. 2008; Ferrara et al. 2010) (Fig. 38.1c).

38.3 RPE Lipofuscin and BVMD

There have been numerous reports indicating that RPE lipofuscin is increased in BVMD. Some of these human studies have been based on non-quantitative analysis (Frangieh et al. 1982; Weingeist et al. 1982) while others acquired measurements from electron micrographs (O'Gorman et al. 1988) or biochemical analysis (Bakall et al. 2007). In some BVMD patients non-lesion posterior fundus exhibited AF levels within 2 standard deviations of age-matched controls while in most cases the entire fundus was reported to display abnormally intense AF (von Ruckmann et al. 1997a).

38.4 Quantitative Fundus Autofluorescence in Best Vitelliform Macular Dystrophy

Underlying disease processes in BVMD are poorly understood and a pathway leading to increased RPE lipofuscin formation is not obvious. Thus, we undertook a disciplined approach to measuring the intensity of fundus AF outside the lesion area. To this end, short-wavelength AF images (488 nm excitation) were acquired with a cSLO (Heidelberg

Spectralis, HRA+OCT; Heidelberg Engineering, Heidelberg, Germany). To enable comparisons amongst patients, image grey levels (GLs) were normalized to the GLs in an internal fluorescent reference (Fig. 38.2b) installed in the instrument and the sensitivity used was within the linear range of the detector ($GL < 175$). Additional protocol details are described in Fig. 38.2 and in published work (Delori et al. 2011).

As shown in Fig. 38.2a, qAF increased with age in both healthy eyes and in non-lesion areas of BVMD retina. Importantly, in all BVMD eyes, qAF values outside the lesion were within normal limits for age. qAF values within the lesion were elevated and the emission spectra were consistent with that of lipofuscin (Duncker et al. 2014).

38.5 What Have We Learned?

By applying the qAF approach to BVMD patients, we found that in fundus areas outside the central lesion, RPE lipofuscin levels are not increased. Thus a generalized increase in RPE lipofuscin is unlikely to contribute to the pathogenesis of BVMD. Except for the area of the lesion and an adjacent transition zone, retinal lamina appeared normal in SD-OCT scans.

The precise role of the BEST1 protein has been difficult to elucidate. Multiple anion channel functions have been attributed to BEST1 including an outward calcium-dependent chloride conductance and bicarbonate efflux (Sun et al. 2002; Rosenthal et al. 2006; Qu and Hartzell 2008; Marmorstein et al. 2009). Due to osmotic forces, the outward flux of chloride and bicarbonate across the basolateral membrane of RPE is followed by fluid transport. Mutations in *BEST1* leading to the loss of anion channel activity and insufficient fluid transport could be the cause of the fluid-filled detachment between photoreceptor cells and RPE that is detected by SD-OCT. Reduced fluid flux is a feature of an induced pluripotent stem cell model of BVMD (Singh et al. 2013). Since RPE lipofuscin is well known to originate from photoreceptor outer segments (Sparrow et al. 2012), the intensely autofluorescent reflective material in the vitelliform lesion likely originates from accumulating outer segment debris within the lesion. Otherwise, increased RPE lipofuscin is unlikely to be a feature of the primary disease process.

Acknowledgments

Supported by National Eye Institute EY024091 and a grant from Research to Prevent Blindness to the Department of Ophthalmology, Columbia University.

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Fig. 38.1.

Multimodal imaging of a BVMD patient (age 14 years) in the vitelliform stage. Fundus autofluorescence (**a**), color fundus photograph (**b**) and horizontal SD-OCT scan (**c**). Corresponding positions in **a**, **b** and **c** are shown as *dashed vertical lines*. The position and horizontal extent of the SD-OCT scan (**c**) is indicated by the *green arrow* in (**a**). **a**. By fundus autofluorescence the foveal lesion exhibits an increased signal. In the SD-OCT image, a *dome-shaped* foveal lesion that includes a hyperreflective component is revealed. The retina appears qualitatively normal outside the lesion. Reflectivity bands in outer retina are attributed to outer nuclear layer (*ONL*); external limiting membrane (*ELM*); ellipsoid region of inner segment (*EZ*); interdigitation zone (*IZ*) and RPE/Bruch's membrane complex (*RPE/Br*) (Staurenghi et al. 2014). The area of separation is between bands attributable to EZ and RPE/Br

Fig. 38.2.

Quantitative fundus autofluorescence (qAF). qAF was calculated from images (488 nm excitation) obtained from 27 eyes of 16 BVMD patients (*red circles*) and 277 healthy subjects reported previously (Greenberg et al. 2013) (*black circles*) and plotted as function of age (**a**). qAF was measured in pre-determined circularly arranged segments (*red*, 8 segments/ring). (**b**) Mean non-lesion qAF plotted (**a**) are based on values obtained from outer ring (**b**). Mean non-lesion qAF (*solid black line* in **a**) of healthy subjects is also shown. The segments were scaled to the distance between the temporal edge of the optic disc (*white vertical line*) and the center of the fovea (*white cross*) (**b**). Details of image acquisition and analysis are published (Delori et al. 2011)