Multimodal Imaging Including Spectral Domain OCT and Confocal Near Infrared Reflectance for Characterization of Outer Retinal Pathology in Pseudoxanthoma Elasticum

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PURPOSE. To investigate the value of multimodal confocal scanning laser ophthalmoscopy (cSLO) for phenotyping fundus lesions in patients with pseudoxanthoma elasticum (PXE) and to correlate these findings with morphologic alterations detected by spectral domain optical coherence tomography (SD-OCT).

METHODS. Imaging was performed with a combined SD-OCT-cSLO system (Spectralis HRA-OCT; Heidelberg Engineering, Heidelberg, Germany). OCT scans were placed at locations of interest on near-infrared (NIR) reflectance, fundus autofluorescence (FAF), and fluorescein angiography (FA) images. The instrument allowed for exact topographic correlation of findings on OCT and cSLO images.

RESULTS. NIR reflectance imaging showed the highest sensitivity to detect angioid streaks and peau d’orange compared to FAF or FA. On OCT scans, angioid streaks reliably showed breaks in Bruch’s membrane. Peau d’orange was associated with alternating reflectivity within Bruch’s membrane. Characteristic mid-peripheral chorioretinal atrophies showed hyporeflective spaces involving the outer neurosensory retina. In eyes with pattern dystrophy like alterations, subneurosensory accumulation of material was observed within areas of increased FAF.

CONCLUSIONS. SD-OCT in combination with cSLO imaging using NIR light locates the primary pathologic formations of angioid streaks and peau d’orange in Bruch’s membrane. NIR reflectance imaging may be superior for detecting PXE-related fundus lesions at the level of Bruch’s membrane, because the blue laser light that is used in FAF and FA is markedly absorbed by the pigment epithelium and therefore may only detect alterations if this cell layer is also affected. The findings indicate that multimodal cSLO and SD-OCT imaging of the outer retina allows for screening of PXE related retinal alterations. (Invest Ophthalmol Vis Sci. 2009;50:5913–5918) DOI:10.1167/iovs.09-3541

Pseudoxanthoma elasticum (PXE) is a rare systemic disease mainly affecting the cardiovascular system, skin, and eyes with a variable phenotype.1,2 The disorder with a prevalence estimated to be 1 in 25,000 to 100,000 is a consequence of mutations in the ABCC6 gene.3 Progressive fragmentation and calcification of elastic fibers in connective tissue result in pathologic changes mostly pronounced in the dermis, Bruch’s membrane, and blood vessels.1,2 Characteristic lesions at the fundus are angioid streaks, peau d’orange, secondary choroidal neovascularization, diffuse chorioretinal atrophy, and chorioretinal atrophic spots in the midperiphery, often with comet tails pointing toward the posterior pole.1,2 Fundus lesions similar to those observed in pattern dystrophies have also been described.4 So far, phenotypic investigation has been based mainly on standard imaging methods such as ophthalmoscopy and fundus photography.5 However, fundus autofluorescence (FAF) imaging was recently found to reveal characteristic changes in patients with PXE.5,6 Other topographic fundus imaging modalities such as near-infrared (NIR) reflectance have never been used, and the relative value of specific imaging modalities for phenotyping retinal lesions in PXE remains unknown. It also remains to be determined whether such multimodal fundus imaging allows sensitive detection and characterization of fundus lesions in PXE.

Histologic studies of fundus changes due to PXE are rare, and the underlying histologic alterations of such fundus lesions remain largely unknown.7–15 High-resolution spectral domain optical coherence tomography (SD-OCT) has recently become available and offers a quasi histologic assessment of the posterior ocular fundus in vivo.13–15 Systems combining SD-OCT with a confocal scanning laser ophthalmoscope (cSLO) allow simultaneous recordings of cross-sectional OCT images with various topographic imaging modes such as NIR reflectance, FAF, and fluorescein angiography (FA) of the cSLO. Exact alignment of the SD-OCT scans with the topographic cSLO images permits to correlate quasi in vivo histology with pathologic features observed on FAF, reflectance, and angiography images.

The purpose of this study was to investigate PXE-related fundus lesions using multimodal cSLO imaging and to study whether SD-OCT reveals underlying morphologic alterations that are detected by specific topographic fundus imaging modalities.

METHODS

Fifty-six eyes of 28 patients (8 male and 20 female patients) with fundus abnormalities due to PXE were investigated. All patients were seen in the outpatient clinic at the Department of Ophthalmology, University of Bonn, which is a tertiary PXE-referral center in Germany. The diagnosis of PXE was positively confirmed by a skin biopsy, genetic analysis, or characteristic funduscopic pathologic changes in combination with further systemic manifestations typical for PXE.

All patients underwent a complete ophthalmic examination, including best corrected visual acuity, indirect ophthalmoscopy, fundus pho-
ography (FF450; Carl Zeiss Meditec, Jena, Germany), and combined cSLO and SD-OCT imaging (Spectralis HRA-OCT, Heidelberg Engineering, Heidelberg, Germany).

The study was in compliance with the tenets of the Declaration of Helsinki, and informed consent was obtained from every patient.

**Fundus Imaging**

The cSLO unit of the Spectralis HRA-OCT is similar to the widely used HRA2 (Heidelberg Engineering, Heidelberg, Germany) and uses an optically pumped solid state laser source to generate the blue light excitation wavelength of 488 nm for FA and FAF images. Recorded emission wavelengths are limited by a barrier filter to wavelengths between 500 and 700 nm. A diode laser source of 820 nm wavelength is used for NIR reflectance recordings. With confocal image acquisition, light from a conjugate plane of interest is detected by the image sensor, permitting suppression of light from planes anterior and posterior to the plane of interest and resulting in high-contrast fundus images.

The high-resolution SD-OCT has a 7-μm optical depth resolution and a 14-μm lateral optical resolution. The system acquires 40,000 A-scans per second. In the present study, the B-scan angle was set to 30° with 768 A-scans per B-scan, resulting in a lateral resolution of 11 μm/pixel and a scan rate of 50 B-scans per second.

Using automated eye tracking and image alignment based on cSLO images, the software allows averaging a variable number of single images in real time (ART, [Automatic Real Time] Module; Heidelberg Engineering). The OCT B-scan is then repositioned in the moving eye and thus stabilized and frozen at the selected retinal location. The software computes and compensates for movements between single B-scan images caused by eye movements. Averaging live B-scans improves the signal-to-noise ratio and therefore enhances image quality with increased B-scan contrast and detail.

**RESULTS**

**Angioid Streaks**

On SD-OCT scans, the pathology of angioid streaks was localized to the Bruch’s membrane-retinal pigment epithelium (RPE) complex (Fig. 1). The width of the gap in Bruch’s membrane was variable and the two breaking edges were usually in one horizontal plane (especially when gaps were narrow), but could also be displaced. The RPE overlying breaks in Bruch’s membrane could seem unaffected, but often appeared altered, focally detached, or absent. After growing from the choroidal space through the gaps in Bruch’s membrane, fibrous tissue may be present underneath the neurosensory retina. Asterisk: outer nuclear layer. Arrowhead: junction between outer and inner photoreceptor segments.

**Figure 1.** Multiple examples for breaks in Bruch’s membrane in patients with PXE. Vertical arrows: small breaks; inclined arrows: larger breaks. The RPE epithelium overlying Bruch’s membrane may appear virtually normal, altered, focally detached, or absent. After growing from the choroidal space through the gaps in Bruch’s membrane, fibrous tissue may be present underneath the neurosensory retina. Asterisk: outer nuclear layer. Arrowhead: junction between outer and inner photoreceptor segments.
this eye were not visible on funduscopy or NIR reflectance imaging.

Comparison of images recorded by confocal NIR reflectance, FAF, and FA revealed the most reliable detection of angioid streaks in the NIR mode (Fig. 3). Breaks in the Bruch’s membrane–RPE complex were usually present on SD-OCT scans, even if angioid streaks were visible only on NIR reflectance but not on FAF imaging or FA (Figs. 3A–H). In some cases, the break appeared to be restricted to the outer part of the Bruch’s membrane–RPE complex. The width of the angioid streak on NIR reflectance imaging corresponded to the defect in Bruch’s membrane visible on OCT imaging. If visible on FAF and FA, the width of the streaks could vary in those imaging modalities depending on concomitant structural alterations, such as accompanying RPE atrophy or hyperplasia. If viable RPE was present surrounding a streak visible on FAF images, contrast compared with background was usually better than in the FA or NIR reflectance images.

Peau d’Orange

With the cSLO, visualization of peau d’orange was best using confocal NIR reflectance imaging (Figs. 4, 5). On confocal NIR reflectance, peau d’orange appears with small flecks of lower reflectivity compared with background which can be confluent, predominantly at the temporal posterior pole. FAF as well as FA were usually unremarkable or rarely revealed a fine mottling within areas of marked peau d’orange. Compared to flecks of lower reflectivity, areas of higher reflectivity on NIR reflectance showed an increased signal at the outer Bruch’s membrane–RPE complex on SD-OCT scans (Fig. 5).

Peripheral Chorioretinal Atrophy

Chorioretinal atrophic spots usually occur in the midperiphery and were detected by all imaging modes used in this study. They showed increased reflectance on NIR images and an increased signal on FAF and late-phase FA. This increased signal could be blocked by focal hyperpigmentations. Frequently, the comet’s tail also showed an increased FAF signal. Since early angiographic sequences were always obtained from the posterior pole (30° × 30°), we do not know the appearance of salmon spots in the early angiographic phase.

SD-OCT scans through lesions at the peripheral fundus are difficult to record. Therefore, only a few scans through salmon spots are available. These showed hyporeflective spaces involving the outer neurosensory retina with a slightly hyperreflective inner lining and focal debris-like deposits just above the RPE level (Fig. 6).

**Figure 2.** Fundus photograph (A) and near-infrared reflectance image (B) of a patient with PXE showing marked atrophic alterations. Breaks in Bruch’s membrane were only visible on the OCT scan (C).

**Figure 3.** Angioid streaks in PXE may be visible on the NIR reflectance (A; black arrows) but not on FAF (B) or FA (C). SD-OCT reveals breaks in Bruch’s membrane exactly corresponding to the angioid streaks visible on NIR reflectance imaging. Below the SD-OCT scans are magnified cutouts of the area marked by the white bars in (A–C) and by the black squares in (D). The RPE appears to cover the break in Bruch’s membrane below. (E–H) Similar findings in a larger streak that passes through the central posterior pole. (I–L) Streaks that are also clearly visible on FAF and FA imaging. On SD-OCT scans (L), the breaks appeared larger or with a small pigment epithelial detachment. Asterisk and arrowhead are as in Figure 1.
Pattern Dystrophy-like RPE Changes

In two patients with fundus findings similar to pattern dystrophy, areas with an increased autofluorescence signal were associated with material deposited below the neurosensory retina (Fig. 7). It was not possible to distinguish whether this material was located within the RPE layer, which may be focally distended, or just below or above the RPE.

DISCUSSION

It is known from histopathologic studies that the primary alteration at the fundus in PXE is located in Bruch’s membrane.7–12 In our study, confocal NIR reflectance imaging at 820 nm turns out to be an excellent noninvasive method for imaging such abnormalities in Bruch’s membrane in vivo. Elsner et al.16 were the first to suggest this imaging technique for visualizing deep retinal and subretinal structures. In retinal imaging, confocality enables visualization of alterations of a given plane with improved contrast and detail. Scattered light is not detected by the cSLO but leads to decreased image quality when a fundus camera is used. Therefore, cSLO NIR reflectance fundus images are mainly affected by reflection and absorption. Moreover, melanin only mildly absorbs NIR light, leading to good visibility of structures below the RPE and to less interindividual variation depending on overall fundus pigmentation.16

In histopathologic studies, eyes of patients with PXE revealed the common finding of breaks in Bruch’s membrane with a topographic arrangement consistent with that of angioid streaks.7–12 Based on clinical observations and discussing physical–optical phenomena, Koffler17 was the first to suggest this anatomic context. We used topographic cSLO imaging for detection of angioid streaks and simultaneous SD-OCT imaging for quasi in vivo histologic assessment. This study provides the first direct evidence that breaks in Bruch’s membrane are indeed the underlying pathology of angioid streaks. The good visibility of the broken edges on SD-OCT scans may be explained by the considerable thickening and calcification of Bruch’s membrane in patients with PXE.7,8

On confocal NIR reflectance imaging, breaks in Bruch’s membrane appear darker compared with the surrounding fundus reflex. Similarly, anatomic structures with interruption of Bruch’s membrane, such as at the optic disc rim, show a decrease in reflectance.16 This decrease suggests that Bruch’s membrane is a significant reflector for NIR light at the fundus, and it may even be increased in patients with PXE due to the calcification of Bruch’s membrane. The superior visibility of peau d’orange with confocal NIR reflectance also locates the primary underlying disease below the RPE. Assuming that calcification of Bruch’s membrane further increases the reflectivity on NIR reflection, the darker flecks in peau d’orange would represent areas still spared from calcification. The herein presented findings on SD-OCT scans (Fig. 5) support this hypothesis. The neurosensory retina overlying peau d’orange revealed no characteristic pathologic formations on SD-OCT scans. Accordingly, retinal sensitivity and dark adaption characteristics were reported not to be affected in areas of peau d’orange.18

The poorer detection of angioid streaks on FAF and FA imaging may be explained by the stronger absorbance of
monochromatic light of 488 nm by melanin and lipofuscin granules in the RPE, which can be preserved overlying angioid streaks. Therefore, on FAF and FA images, angioid streaks may only become visible with concomitant alterations of the RPE, such as loss of pigment granules or ruptures in the cell layer as described in histologic studies. RPE damage may then extend beyond the lateral margins of breaks in Bruch’s membrane. Further studies are necessary to assess whether these alterations of the RPE layer may have functional (e.g., localized scotomas) and/or prognostic (e.g., on the development of choroidal neovascularization) consequences.

SD-OCT may serve as a tool to investigate PXE-related abnormalities. For instance, subneurosensory fibrous tissue and breaks in Bruch’s membrane in areas of retinal atrophy were not consistently identified on cSLO images or fundus photography, but were detected by SD-OCT. Dislocation of fragmented pieces of Bruch’s membrane from a common plane was previously interpreted as fixation artifacts in histologic studies. However, in vivo SD-OCT revealed that such displacements of Bruch’s membrane fragments are a real feature of PXE. In patients with very advanced fundus changes such as large areas of atrophy and fibrotic alterations, the underlying primary pathologic characteristic—breaks in a thickened and degenerated Bruch’s membrane—cannot be detected on FA, fundus photography, NIR, or FAF imaging.7 Hu et al. stated that RPE atrophy may “become so extensive around the disc and in the macular area that one can no longer see any signs of angioid streaks” and that this may complicate the ophthalmic diagnosis of PXE. Noninvasive SD-OCT imaging may still detect the characteristic breaks in Bruch’s membrane and therefore allows uncovering the pathogenic context.

Pattern dystrophy–like changes have been reported to be associated with PXE. A histologic study of an eye with pattern dystrophy due to a mutation in the RDS/peripherin gene revealed a thickened Bruch’s membrane, a greatly distended RPE due to excessive accumulation of lipofuscin and partially atrophic photoreceptors. SD-OCT imaging in a patient with PXE with apparent pattern dystrophy-like changes revealed similar findings: a thickened Bruch’s membrane–RPE complex with corresponding increased FAF signal indicating accumulation of autofluorescent material (Fig. 7). In PXE, a generalized retinal and RPE alteration due to the abnormal underlying Bruch’s membrane have been suggested, and pattern dystrophies may present with subnor-

FIGURE 6. SD-OCT scans through peripheral chorioretinal atrophic spots in two patients with PXE. (A, B) FAF shows an increased signal within the area of the atrophy, presumably originating from the sclera. OCT scans (C, D) revealed hyporeflective spaces of the outer neurosensory retina with a slightly hyperreflective inner lining and focal debris-like deposits just above the RPE-level.

FIGURE 7. Pattern dystrophy-like fundus changes in a patient with PXE. Areas with an increased autofluorescence signal are associated with deposit-like alterations below the neurosensory retina. The outer nuclear layer overlying these lesions was decreased in thickness.

References