Quantitative Fundus Autofluorescence in Pseudoxanthoma Elasticum

Martin Gliem,1,2 Philipp L. Müller,1,2 Johannes Birtel,1,2 Myra B. McGuinness,3 Robert P. Finger,1 Philipp Herrmann,1,2 Doris Hendig,4 Frank G. Holz,1,2 and Peter Charbel Issa1,2,5

1Department of Ophthalmology, University of Bonn, Bonn, Germany
2Center for Rare Diseases Bonn (ZSEB), University Hospital of Bonn, Bonn, Germany
3Centre for Eye Research Australia, University of Melbourne, Melbourne, Australia
4Institute for Laboratory and Transfusion Medicine, Heart and Diabetes Center North Rhine-Westphalia, University Hospital of the Ruhr University of Bochum, Bad Oeynhausen, Germany
5Oxford Eye Hospital, Oxford University Hospitals NHS Foundation Trust and the Nuffield Laboratory of Ophthalmology, Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom

Correspondence: Peter Charbel Issa, Oxford Eye Hospital, John Radcliffe Hospital, Headley Way, Oxford OX3 9DU, UK; study-enquiry@outlook.com.

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PURPOSE. To quantify lipofuscin-associated fundus autofluorescence in patients with pseudoxanthoma elasticum (PXE), a model disease for Bruch’s membrane pathology.

METHODS. In this prospective, monocenter, cross-sectional case-control study, 49 patients with PXE (mean age: 46 years, range 18–62) underwent quantitative fundus autofluorescence (qAF) imaging with a modified scanning laser ophthalmoscope containing an internal fluorescent reference for normalization of images. The mean qAF values of a circular ring centered on the fovea (qAF8) were measured and compared to 108 healthy controls (mean age 40 years, range 18–64).

RESULTS. Overall, patients with PXE showed lower qAF8 values compared to controls (difference from controls −23%, 95% confidence interval [CI] −29% to −16%, P < 0.001). The reduction was most pronounced in patients older than 40 years (−30%, 95% CI −36% to −25%, P < 0.001) and was negatively correlated with the extent of Bruch’s membrane calcification (r = −0.49, 95% CI: −0.67 to −0.22). The topographic distribution revealed a greater reduction of qAF8 values toward the optic disc than temporally compared to controls (P < 0.001). The phenotype of patients with reduced qAF8 values was characterized by pattern-dystrophy-like changes (71%; 10 of 14), reticular pseudodrusen (71%; 10 of 14) and limited areas of atrophy (29%, 4 of 14).

CONCLUSIONS. Reduced qAF8 values are a characteristic finding in patients with PXE, indicating that Bruch’s membrane disease may result in a modification of the accumulation, distribution, or composition (or a combination thereof) of lipofuscin in retinal pigment epithelial cells.

Keywords: pseudoxanthoma elasticum, Bruch’s membrane, autofluorescence, quantitative fundus autofluorescence, retinal pigment epithelium.

Quantitative fundus autofluorescence (qAF) may be used as a surrogate marker for lipofuscin accumulation in the RPE.1 Lipofuscin has been associated with toxic effects on the RPE2,3 and is therefore considered as an important pathogenic factor in various retinal diseases, including AMD.4 Using qAF imaging, we recently showed that patients with early and intermediate AMD have normal or low qAF levels when compared to age-related controls.5 The reason for this rather unexpected finding has as yet remained a matter of hypothesis and speculations.

Among other possible explanations, dysfunction of the choroid-Bruch’s membrane–RPE complex was discussed, which could possibly lead to slowing of the visual cycle.5 Reduced turnover of vitamin A derivatives would hence result in reduced lipofuscin accumulation and lower qAF measures. An important factor in this context could be pathology of Bruch’s membrane, which is known to occur in AMD.5 A diseased Bruch’s membrane could then lead to an increased resistance for vitamin A molecules to enter the visual cycle from the choroid. Furthermore, an altered metabolism of RPE cells overlying a pathologic Bruch’s membrane could interfere with normal visual cycle dynamics.

Pseudoxanthoma elasticum (PXE; OMIM# 264800) may be used as a model disease to study the effects of a primary ocular pathology in Bruch’s membrane. The ocular phenotype of PXE is characterized by an early onset calcification of Bruch’s membrane, which starts posteriorly close to the optic disc and spreads toward the periphery over time. The transition zone between the centrally calcified and peripherally noncalcified Bruch’s membrane forms a speckled area, the so-called “peau d’orange,” which is usually most prominent temporally.7,8 Secondary to the calcification of Bruch’s membrane, other PXE-related fundus changes may develop, including angiod streaks; reticular pseudodrusen (RPD); choroidal neovascularization (CNV); and areas of atrophy.8–10 Overall, the ocular phenotype in patients with PXE shows similarities with AMD, suggesting...
common pathogenic pathways at the level of Bruch’s membrane.

The aim of this study was to further explore the hypothesis that Bruch’s membrane changes could result in reduced lipofuscin-related qAF measures. For this purpose, we investigated the impact of a diseased Bruch’s membrane on lipofuscin levels within the RPE using qAF imaging in patients with PXE.

**Materials and Methods**

This prospective cross-sectional case-control study was conducted in adherence to the tenets of the Declaration of Helsinki. Institutional review board approval (Ethikkommission, Medizinische Fakultät, Rheinische Friedrich-Wilhelms-Universität Bonn) and patient consent were obtained. Patients were recruited from a dedicated clinic for rare retinal degenerations at the Department of Ophthalmology, University of Bonn, which serves as the German national referral center for PXE.

Inclusion criteria were the diagnosis of PXE based on clinical findings typical for PXE including peau d’orange, angioid streaks, and peripheral comet lesions as well as genetic testing as described previously and/or histopathologic findings in skin biopsies.

Exclusion criteria were aged <18 and >65 years, any pathology affecting the ocular media such as corneal opacities, cataract unusual for age, pseudophakia, vitreous opacities, pupil diameter <7 mm, CNV-related changes (e.g., fibrosis, intra/subretinal fluid) within the area of qAF measurement, unstable fixation, refractive error ±6 diopters (spherical equivalent), atrophy >50% within the area of measurement or any other pathology of the macular region not related to PXE.

**Image Recordings and Analysis**

Prior to imaging, pupils were dilated by instillation of 0.5% tropicamide and 2.5% phenylephrine. All subjects underwent a standardized imaging protocol consisting of fundus photography (Visucam; Carl Zeiss Meditec, Jena, Germany); spectral-domain optical coherence tomography (SD-OCT); near-infrared (NIR) reflectance; and fundus autofluorescence (AF) imaging with a confocal scanning laser ophthalmoscope (486-nm excitation, Spectralis HRA+OCT, Heidelberg Engineering, Heidelberg, Germany).

QAF imaging was performed as described previously according to the method developed by Delori et al. Briefly, an OCT device (Spectralis HRA; Heidelberg Engineering) was equipped with an internal fluorescent reference to account for fluctuations in laser power and differences in detector sensitivity. After switching to the qAF mode (486-nm excitation and 500–680 nm detection) focus and alignment were adjusted to obtain a maximum and uniform signal. The visual pigment sensitivity. After switching to the qAF mode (486-nm excitation, Spectralis HRA+OCT, Heidelberg Engineering). The overall qAF value was computed as the mean of the qAF values of the eight segments (qAF8). QAF8 values were compared to 108 healthy controls of Caucasian ethnicity (age range, 18–64 years) without any eye disease. More central and peripheral segments were not analyzed due to the decreasing reliability of measurements (e.g., macular pigment within central segments and decreasing field uniformity and repeatability within peripheral segments).

Color-coded qAF maps were computed based on pixel-wise transformation of qAF values into colors using a custom-made extension of the ophthalmic imaging software (Heidelberg Engineering).

To assess the extent of Bruch’s membrane calcification, the temporal eccentricity of peau d’orange (or “eccentricity of the leading edge of Bruch’s membrane calcification”: EBC) was used, although its formal validation as a surrogate marker for disease progression is pending. The EBC was shown to be associated to other PXE-related findings, such as angioid streaks that only occur within calcified areas, and reticular pseudodrusen (RPD).

The EBC was measured based on 30° NIR reflectance images (due to the higher sensitivity compared with fundus color images to detect peau d’orange) using the integrated measurement tool of the ophthalmic imaging software (Heidelberg Engineering). Measurement was performed from the temporal edge of the optic disc to the most central border of peau d’orange in the temporal (mid-) periphery (Fig. 1). If peau d’orange was not
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**RESULTS**

The study included 49 individuals with PXE (36 female, 13 male) with a mean age of 46 years (range, 18–62 years) and 108 controls (72 female, 36 male) without any eye disease (mean age 40, range: 18–64 years). Molecular genetic testing revealed two disease-causing ABCC6-mutations in 34 patients, and one disease-causing mutation in seven patients. In another seven patients, PXE was confirmed by positive histopathologic findings in skin biopsies. The diagnosis was based on clinical findings alone in 1 patient.

**Quantitative Autofluorescence**

Measurement of qAF₈ revealed reduced values within the age-related 95% prediction interval (PI) of controls in 34 patients (69%). QAF₈ was below the 95% PI in 14 (29%) and above in one (2%) patient (Fig. 2A). After adjusting for age statistical analysis revealed overall reduced qAF₈ values in PXE patients compared to controls (difference from controls –23%, 95% CI –29% to –16%, \( P < 0.001 \)).

In younger patients below 40 years of age (\( n = 13 \)), QAF₈ was within the 95% PI in 12 (92%) patients and slightly above in one patient (Fig. 2A). Accordingly, statistical analysis showed no significant difference compared to controls (difference from controls –3%, 95% CI –14 to –11%, \( P = 0.728 \)). In patients 40 years and older (\( n = 36 \), 14 (39%) had qAF₈ values below the 95% PI and statistical analysis showed an overall significantly lower qAF₈ level for cases compared to controls (difference from controls –30%, 95% CI –36% to –23%, \( P < 0.001 \)).

**Statistical Analysis**

Data were collected and illustrated using spreadsheet (Microsoft Corp.) and graphing software (GraphPad Prism version 6; GraphPad Software, Inc., La Jolla, CA, USA). Statistical analysis was performed using statistical software (SPSS version 20.0; IBM Corp., Armonk, NY, USA; and Stata/IC version 13.1; College Station, TX, USA). Prediction intervals (PI) were calculated assuming a linear relationship between the log of age and the log of qAF₈ units for all control subjects. Linear regression of the log of qAF₈ was used to compare patients to controls adjusting for age statistical analysis showed no significant difference compared to controls (difference from controls –3%, 95% CI –14 to –11%, \( P = 0.728 \)). In patients 40 years and older (\( n = 36 \), 14 (39%) had qAF₈ values below the 95% PI and statistical analysis showed an overall significantly lower qAF₈ level for cases compared to controls (difference from controls –30%, 95% CI –36% to –23%, \( P < 0.001 \)).
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A possible biomarker for the extent of PXE-related ocular effects is the eccentricity of the leading edge of Bruch’s membrane calcification (EBC; see methods).\(^7\)\(^8\) For a better visualization of a possible association between EBC and qAF\(_8\) values, eyes were differentiated into those with limited (blue dots) and those with more widespread (red dots) Bruch’s membrane calcification, arbitrarily defined as an EBC below or above 6000 \(\mu\)m (approximately 20\(^{\circ}\), meaning peau d’orange only visible at the temporal border of 30\(^{\circ}\) NIR reflectance images, Fig. 2B). Based on this grouping widespread calcification was present in all eyes (14 of 14) with qAF\(_8\) values below the 95\% PI (mean EBC = ± SD; 8085 ± 1212 \(\mu\)m), but in only 40\% (14 of 35) of eyes with normal qAF\(_8\) levels (5090 ± 2613 \(\mu\)m; \(P < 0.001\)). The graph also illustrates older age not necessarily being associated with more extensive peau d’orange and/or low qAF due to the variable disease manifestation in PXE.

To correlate qAF\(_8\) values with EBC, qAF\(_8\) z-scores were calculated. The z-score is independent from age, as it represents the number of SDs of a qAF\(_8\) value from the mean of age-related controls. QAF\(_8\) z-scores were negatively correlated with EBC (\(r = -0.47, 95\%\ CI = -0.67; -0.22, P < 0.001\), Fig. 3A). Plotting EBC over age illustrated that calcification increased with aging, although with substantial variability. Patients with a z-score 2 SDs below average are usually those at older age with more progressed calcification (Fig. 3B).

The overall topographic distribution of qAF values was comparable between controls and PXE patients with highest values in the superotemporal/temporal and lowest values in the nasal sector. However, reduced qAF\(_8\) levels in patients compared to controls were more pronounced close to the optic disc compared with eccentric (temporal) areas indicating a topographic association with the calcification of Bruch’s membrane. This is in line with an earlier and more extensive calcification of Bruch’s membrane in the papillomacular region, from where it spreads centrifugally toward the periphery. To further illustrate this finding, individual qAF levels from the temporal and nasal segments were plotted for patients and controls (Figs. 4D, 4E). Quantitative analysis revealed a greater difference of qAF values between patients and controls in the nasal segment (24\% difference from controls, 95 CI: 21\%–27\%) than in the temporal segment (9\% difference from controls, 95 CI: –7\% to –10\%). This difference in effect size between segments was significant (interaction term: 0.187 log qAF units, 95\% CI: 0.142–0.232, \(P < 0.001\), Figs. 4D, 4E).

Eyes of PXE patients with reduced qAF values showed characteristic fundus changes (Figs. 5D–F), including eccentric EBC; pattern dystrophy-like changes (71\%, 10 of 14); reticular pseudodrusen (71\%, 10 of 14); and limited atrophy (29\%, 4 of 14). Such fundus features were significantly less frequent in patients with normal qAF values (pattern dystrophy-like changes: 11 of 35, 31\%; \(P = 0.023\); reticular pseudodrusen: 9 of 35, 26\%; \(P = 0.008\); atrophy: 2 of 35, 6\%; \(P = 0.048\), Figs. 5A–C). Notably, eyes with advanced fundus alterations such as widespread atrophy or fibrosis were not included in the study.

**DISCUSSION**

This study indicates that a diseased Bruch’s membrane in patients with PXE is associated with reduced qAF measures. When corrected for age, there was an inverse relationship between qAF levels and more advanced ocular manifestations of PXE, which include the extent of Bruch’s membrane calcification, pattern dystrophy-like changes, reticular pseudodrusen, and atrophy. This finding across the entire cohort is supported by the topographically resolved analysis within eyes.
as well as patients with Sorsby fundus dystrophy and late-onset Consistently, patients with PXE (own unpublished observations) include an increased barrier function of Bruch’s membrane as well as concomitant alterations of the inner choroid resulting in a result of a diseased Bruch’s membrane. Possible explanations include an increased barrier function of Bruch’s membrane as well as concomitant alterations of the inner choroid resulting in reduced availability of vitamin A and impaired RPE metabolism.

Reduced qAF levels in patients with PXE may suggest reduced lipofuscin content in the RPE. Although pathophysiologic considerations remain speculative, this finding is in agreement with a hypothesized slowing of the visual cycle as a result of a diseased Bruch’s membrane. Possible explanations include an increased barrier function of Bruch’s membrane as well as concomitant alterations of the inner choroid resulting in reduced availability of vitamin A and impaired RPE metabolism. Consistently, patients with PXE (own unpublished observations) as well as patients with Sorsby fundus dystrophy and late-onset retinal degeneration (LORD) which demonstrate pathophysiologic overlap at the level of the Bruch’s membrane-RPE interface, typically show prolonged dark adaptation consistent with a slowdown of the visual cycle. To further confirm this hypothesis investigation of qAF levels in patients with Sorsby fundus dystrophy or LORD would be meaningful.

A diseased Bruch’s membrane may also lead to RPE atrophy. This process might not only result in areas of complete RPE loss, but also in reduced cell density of the remaining RPE layer. The latter situation would offer an alternative explanation for low qAF levels even if the lipofuscin content in individual cells would be similar to normal controls.

In a previous interpretation of similar findings in AMD patients, additional explanations (or a combination thereof) for lower qAF measurements included different packing, density or distribution of lipofuscin within RPE cells, altered composition of fluorophores or blockage by intracellular (e.g., melanin) or extracellular structures (e.g., subretinal drusenoid deposits). Further studies focusing on the exact composition and distribution of fluorophores are therefore needed.

Moreover, measurements could also be affected by PXE-related pathology of the ocular fundus. To overcome this, patients with advanced CNV, fibrosis, or atrophy within the area of measurement were excluded herein. In addition, smaller areas of atrophy (<50% of the area) or pronounced angioid streaks were excluded by histogram analysis. Mild changes such as subtle angioid streaks, pattern dystrophy-like changes or reticular pseudodrusen could have impacted qAF measurements, although these would unlikely explain the effect size observed in older PXE patients.

The observations of this study may be of wider interest when PXE is considered as a model disease for Bruch’s membrane pathology. For instance, AMD has a complex pathophysiology and the reasons for the recently reported decreased qAF remain to be determined. Among other contributing pathways, it is thought that changes of Bruch’s membrane play an important role in the pathogenesis of AMD, including thickening and calcification, deposition of advanced glycation end products, and lipid accumulation.

Furthermore, AMD and PXE-related ocular alterations share phenotypic similarities consistent with common pathogenic pathways at the level of Bruch’s membrane. As the current study suggests an association between Bruch’s membrane disease and lipofuscin-related qAF levels, one could speculate that Bruch’s membrane pathology might at least partially contribute to the reduced qAF levels observed in patients with AMD. Interestingly, prolonged dark adaption has also been reported in patients with AMD. Therefore, impairment of visual cycle dynamics could also be a coherent explanation for reduced lipofuscin accumulation in AMD, although further studies are needed to confirm this hypothesis.

**Figure 5.** Association of phenotype and quantitative fundus AF in PXE. The graph represents the qAF values and the extent of temporal peau d’orange as shown in Figure 2. The images show representative examples of certain subgroups using NIR reflectance (left) and fundus AF images (right). Note that the gray levels of the AF images do not represent AF signal intensity (qAF), which was calculated based on comparison with the gray level of a simultaneously recorded AF reference. Younger patients typically presented only mild changes like angioid streaks and relatively central peau d’orange (A). Midaged patients with normal qAF values typically showed mild fundus changes and less progressed peau d’orange (B). Older patients, again with normal qAF values, frequently had only mild fundus changes like subtle pattern dystrophy-like changes and relatively central peau d’orange (C). Patients with reduced qAF values typically showed reticular pseudodrusen (D), central pattern dystrophy-like changes (E, F); and areas of atrophy (F, arrow), as well as progressed peau d’orange (graph, peau d’orange marked in blue on NIR reflectance images).
In conclusion, this study provides evidence that pathologic changes of Bruch’s membrane may be associated with reduced qAF, indicating reduced lipofuscin levels within the RPE. These findings do not only expand the pathophysiologic understanding of PXE, but also of multifactorial diseases with pathologic changes of Bruch’s membrane including AMD.

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