Frequency, Phenotypic Characteristics and Progression of Atrophy Associated With a Diseased Bruch’s Membrane in Pseudoxanthoma Elasticum

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Purpose. To characterize atrophy of the outer retina and the retinal pigment epithelium in patients with pseudoxanthoma elasticum (PXE).

Methods. In this retrospective cross-sectional study, the frequency and phenotypic characteristics of manifest atrophy were investigated in 276 eyes of 139 patients using color fundus photography, fundus autofluorescence (AF) imaging, and spectral domain optical coherence tomography. Progression rates of atrophy were quantified in eyes with longitudinal AF recordings.

Results. Atrophy was present in 90 eyes (32%; mean age, 60; range, 32–88 years). In 19 eyes (7%; mean age, 56; range, 37–77 years) atrophy occurred without any signs for an active or fibrotic choroidal neovascularization (CNV). The frequency of both, atrophy and CNV, increased with age. In those >60 years of age, atrophy and/or CNV were almost universally present but varied considerably in severity. Eyes with emerging pure atrophy (n = 13, no signs of CNV) showed pattern dystrophy-like changes (100%), reticular pseudodrusen (82%), and reduced choroidal thickness. Advanced atrophy was multifocal, reached beyond the arcades, and was present nasal to the optic disc. The average expansion rate of atrophy was 3.3 ± 1.3 and 1.6 ± 1.1 mm²/year (mean ± SD), in those without or with signs for CNV, respectively.

Conclusions. Atrophy of the outer retina and the retinal pigment epithelium is a common finding in PXE patients characterized by early onset and fast progression with subsequent visual loss independent from CNV. This suggests that atrophy is the natural endpoint of Bruch’s membrane disease. Phenotypic similarities with multifactorial geographic atrophy in age-related macular degeneration suggest common pathogenic pathways at the level of Bruch’s membrane.

Geographic atrophy (GA) is a term commonly used to describe a late-stage manifestation of age-related macular degeneration (AMD). It is characterized by sharply demarcated areas at the posterior pole with atrophy of the photoreceptor layer, the retinal pigment epithelium (RPE), and the choriocapillaris. Despite an enormous socio-economic burden, there is still little knowledge on the pathogenesis of GA. Furthermore, effective treatment options are not yet established. Various possible pathophysiological pathways have previously been considered including increased lipofuscin accumulation, oxidative stress, chronic inflammation, environmental factors, and changes of the choroid and/or the Bruch’s membrane. The potential role of Bruch’s membrane alterations is difficult to assess in vivo as there is currently no imaging modality that would allow detailed analyses of its anatomic structure.

Atrophy of the outer retina and the RPE as a common downstream pathogenetic pathway also occurs in rare monogenic diseases in which pathological changes primarily occur at the level of Bruch’s membrane. The most prevalent of these disease entities is presumably pseudoxanthoma elasticum (PXE), which may serve as suitable model disease to characterize atrophy associated with a primarily diseased Bruch’s membrane.

PXE (OMIM #264800) is caused by biallelic mutations in the ABCC6 gene. Although its exact pathophysiology is unknown, the disease is characterized by systemic calcification and fragmentation of elastic fibers, which also affects the Bruch’s membrane. Resulting characteristic fundus features are angiod streaks, peau d’orange, reticular pseudodrusen (RPD), and early onset choroidal neovascularizations (CNVs).

Atrophy mimicking late AMD has also been described in PXE patients; however, detailed characterization of this disease manifestation has been lacking. The purpose of this study is to investigate the frequency, phenotypic characteristics, and rate of progression of atrophy in patients with PXE.
MATERIALS AND METHODS

Patients

This cross-sectional retrospective study was in adherence to the Declaration of Helsinki. Institutional review board approval (Ethikkommission, Medizinischen Fakultät, Rheinische Friedrich-Wilhelms-Universität Bonn) and patients’ consent were obtained. Patients were recruited from a dedicated clinic for rare retinal diseases at the Department of Ophthalmology, University of Bonn, which is a German national referral center for PXE.

Inclusion criteria were the diagnosis of PXE based on genetic testing as described previously,12,13 histopathologic findings in skin biopsies, and/or presence of characteristic ocular fundus alterations.14 Out of the 302 eyes of 151 patients fulfilling the inclusion criteria, both eyes of 12 patients and one eye of two patients were excluded from further analysis due to insufficient quality of available fundus images, additional retinal pathologies unrelated to PXE, or prior vitreoretinal surgery. Eyes with previous intravitreal injections of vascular endothelial growth factor (VEGF) inhibitors were not excluded.

All patients underwent a complete ophthalmologic examination including best corrected visual acuity (BCVA), slit lamp examination, indirect opthalmoscopy with dilated pupils, and a dedicated imaging protocol. The imaging protocol included fundus color images (Zeiss Visucam, Zeiss, Oberkochen, Germany), fundus autofluorescence (AF), and near infrared (NIR) reflectance images with a confocal scanning laser ophthalmoscope (cSLO; Spectralis HRA, Heidelberg Engineering, Heidelberg, Germany), spectral-domain optical coherence tomography (SD-OCT) images (Spectralis HRA-OCT, Heidelberg Engineering), and in selected cases enhanced depth imaging (EDI) OCT, fluorescein (FA), and indocyanine green angiography (ICG-A).

All eligible eyes were graded for presence or absence of atrophy and CNV (independent from the CNV being active or inactive/fibrotic) at the posterior pole. Grading was based on color, NIR reflectance and AF fundus images, SD-OCT, and in selected cases on FA and ICG-A.

Image Analysis

The progression rate of atrophy was analyzed in eyes for which data were available from at least two independent visits with a minimum interval of 12 months and sufficient image quality. If sufficient images of more than two visits were available, they were also analyzed if the interval between visits was ≥ 2 months. Images were not assessed for progression of atrophy if the area of atrophy extended beyond the image borders. Eyes were analyzed separately based on absence or presence of CNV. To account for the commonly similar disease course between eyes in patients with bilateral atrophy without CNV or bilateral atrophy with CNV, only the right eye was analyzed. In patients with bilateral atrophy but signs for CNV in only one eye, both eyes were analyzed separately.

Evaluation of atrophy progression rates was primarily based on 55° fundus AF images. This lens has the disadvantage of limited validity of measurements outside the central 30° due to increased optic distortion, but enables to also include eyes with larger areas of atrophy. If only 30° images were available for individual visits, they were aligned to the 55° images. Because the current version of a commercially available software for measuring areas of atrophy (RegionFinder, Heidelberg Engineering) is only setup for assessing 30° images, image analysis was performed as follows: Images were exported from the Heidelberg Eye Explorer (HEYEX, Heidelberg Engineering) and were aligned to the most recent image using at least four landmarks and a dedicated software (Multi Modal Mapper15). The area of atrophy was measured manually by delineating the borders of atrophy using the magnetic lasso tool of Adobe Photoshop CS5 (Adobe Systems, San Jose, CA, USA) for automatic detection of contrast edges. In cases with indistinct borders on AF imaging (e.g., in the foveal region), confocal NIR reflectance and SD-OCT images were additionally consulted.16,17 The enclosed area was measured and transformed to mm² by applying the respective scaling derived from the image information tool of the HEYEX software. To exclude contribution of peripapillary atrophy, a circle of 4000 μm diameter was placed centered to the optic disc and excluded from measurement.

Definitions

Atrophy was defined as dropout of the RPE and visibility of the choroidal vessels on fundus color photographs, clearly demarcated hypoautofluorescent areas on fundus AF, and hyperreflective areas on NIR reflectance images. On spectral-domain optical coherence tomography loss of the inner-segment ellipsoid and RPE band, and associated hypertransmission posterior to Bruch’s membrane were considered to support the diagnosis of atrophy. Minimal lesion size was 300 μm diameter based on fundus AF images.

Active or inactive CNV was defined by the following findings: intra- or subretinal fluid on SD-OCT imaging (in selected cases in combination with leakage on angiography), intra- or subretinal hemorrhage, and signs for subretinal fibrosis on fundus color and/or SD-OCT images.

Statistical Analysis

Data were collected and illustrated using Microsoft Excel (Microsoft, Redmond, WA, USA) and GraphPad Prism version 6 (GraphPad Software, Inc., La Jolla, CA, USA). Statistical analysis was performed using SPSS (Version 20.0, IBM Corporation, Armonk, NY, USA). Progression rates of atrophy were compared between groups using two-tailed Student’s t-test after data distribution was confirmed to be Gaussian.

RESULTS

The study included 276 eyes of 139 patients with a mean ± SD age of 50 ± 14 years (range, 14–88 years). The diagnosis of PXE had been confirmed by genetic testing (n = 112), histopathologic findings in skin biopsy (n = 5), or clear clinical evidence for PXE (n = 22).

Frequency of Atrophy in PXE

Image analysis of the 276 eyes of 139 patients revealed atrophy in 90 eyes (32%) of 52 patients (mean age, 60 years; range, 32–88 years), including 19 eyes (7%) of 16 patients (mean age, 56 years; range, 37–77 years) with atrophy only (i.e., without signs or prior therapy for a CNV). Below 40 years of age, all patients had fundus features characteristic for PXE, but atrophy or CNV was rare. Their frequency increased with age and in patients older than 70 years, all eyes had CNV, atrophy, or both. Atrophy without signs for an active or inactive CNV was most frequent between 60 and 70 years of age (15%, 9 of 62 eyes), whereas combined atrophy and CNV was the most common presentation in the elderly (>70 years of age: 69%, 11 of 16 eyes; Figure 1).

Phenotype of Atrophy in PXE

Atrophy without associated CNV was visible on fundus color images with visualization of the underlying larger choroidal vessels. In all but one eye, either focal hyperpigmentations
(42%, 8 of 19 eyes; Figs. 2A, 2D, 2G, arrowheads), yellowish dot-like structures (26%, 5 of 19 eyes), or both (26%, 5 of 19 eyes) surrounding the area of atrophy were visible (Figs. 2A, 2D, 2G). On fundus AF imaging, areas of atrophy showed markedly reduced AF, typically with some remaining low signal with a pattern determined by larger choroidal vessels. All eyes showed fleck- or dot-like, sometimes branching hyperauto-fluorescent patterns surrounding the area of atrophy (Figs. 2B, 2E, 2H). Additional peripapillary atrophy was present in 89% (17 of 19 eyes; Fig. 2). The choroid was markedly thinned (Figs. 2C, 2F, 2I). Of note, angioid streaks were difficult to visualize within areas of atrophy, but remained detectable on SD-OCT images (Figs. 2C, 2F, 2I, arrows).

On SD-OCT, the perilesional zone frequently showed a disruption of the ellipsoid band and hyperreflective spots within the outer retinal layers (Fig. 3). The junctional zone was typically characterized by a sharp break of the RPE and ellipsoid band, a curved external limiting membrane, disappearance of the outer nuclear layer, and hence approximation of the outer plexiform layer (OPL) toward the Bruch’s membrane (topographic sequence from the perilesional zone to the atrophic zone). Within areas of atrophy, the OPL rested on the relatively flat hyperreflective line representing the extension of the RPE/Bruch’s membrane complex band. Below Bruch’s membrane signal transmission was increased (Figs. 2C, 2F, 2I, 3).

For 13 eyes of 8 patients (mean age, 49 years; range, 44–60 years) images before development of atrophy (six eyes of four patients) or with early atrophy (<1 diameter of the optic disc (seven eyes of four patients) and no signs of CNV were available. Typical findings were central pattern dystrophy-like changes, which were best visible as hyperautofluorescent changes on fundus AF images (all 13 eyes), RPD (12 eyes), and...
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Progression of Atrophy in PXE

Twenty eyes of 16 patients were available for evaluating atrophy progression (Fig. 6; Table). Hereof, seven eyes of seven patients showed no signs of CNV (mean age, 52.7; SD ± 5.2; range, 47–62 years; Figs. 6A–C) and 13 eyes of 13 patients had areas of atrophy were surrounded by relatively increased AF on fundus AF images in all eyes with late atrophy (Fig. 5). Analysis of all patients older than 60 years of age revealed large variability of disease severity (Supplementary Fig. S3).

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concomitant active or inactive CNV (52.6 ± 7.2; 37–64 years; Figs. 6D–I). Age (52.7 vs. 52.6 years), atrophy size at baseline (5.5 vs. 4.3 mm²), and mean duration of follow-up (38.7 vs. 36.4 months) were not different between both groups. Atrophy progression was faster in patients without CNV (3.3 mm²/year [SD ± 1.3 mm²; range, 1.0–5.1] Figs. 6A–C, 6J, Table), compared to patients with CNV (1.6 mm²/year [SD ± 1.1 mm²; range, 0.2–3.8; P = 0.01] Figs. 6D–I, 6J, Table). Growth rates, mainly in patients with concomitant active or inactive CNV, could show a high degree of variability (Figs. 6D–I).

DISCUSSION

Atrophy of the outer retina and the RPE is a frequent finding in patients with PXE, a disease leading to severe and early onset calcification of Bruch’s membrane. Because atrophy is almost universally observed in older PXE patients independent from presence or absence of a CNV, it may be seen as the natural endpoint of PXE-associated retinal disease. Thus, atrophy would represent the functionally limiting disease manifestation in PXE patients even if CNV was successfully treated, for example, with anti-VEGF agents. More broadly, considering PXE as a model for Bruch’s membrane disease, atrophy of the outer retina and the RPE may be assumed to be the natural endpoint of a diseased Bruch’s membrane.

Together with previous observations on other PXE-associated fundus features, a typical sequence of disease manifestations may be considered: The leading edge of the initial calcification of Bruch’s membrane is peau d’orange. While the calcification process spreads centrifugally from the posterior pole, angioid streaks (breaks in the brittle, calcified Bruch’s membrane), RPD, and thinning of the choroid follow. Next, development of pattern dystrophy-like alterations with irregular pigmentation may precede the incidence and progression of atrophy. CNV may occur secondary to angioid streaks and may be seen as a complication during the natural disease course.

Possible systemic and/or ocular factors responsible for the variable age of onset of specific fundus changes and for the eventual extend of atrophy are not yet understood. Since these factors might also play a role in other diseases leading to atrophy including GA in AMD, their identification might not only be important for a better pathophysiological understanding, but also for developing novel treatment strategies for GA.

So far, there are only few reports on atrophy without CNV in PXE, including one retrospective case series reporting on eight eyes of five patients. In agreement with the current study, an association of pattern dystrophy-like changes with atrophy was reported. However, RPD and choroidal thickness were not yet assessed. The lower estimated progression rate (1.7 mm²/year; n = 5 patients) and higher frequency (~20%) of pure atrophy reported in that study could be due to a sampling...

**FIGURE 5.** Late stage atrophy in PXE. Widefield fundus color (A, C) and fundus AF images (B, D) of two representative patients (64 and 60 years old) with widespread multifocal atrophy extending beyond the vascular arcades and nasal to the optic disc.
bias in the smaller cohort (21 patients) or due to atrophy assessment on fundus color photographs instead of cSLO images. The diagnosis was primarily based on clinical findings and genetic testing was not performed. Therefore, inclusion of patients with other diseases with a similar phenotype (e.g., β-thalassaemia) could have been possible.

Phenotypic similarities of atrophy in PXE and GA in AMD include patterns of enhanced AF surrounding the atrophy, sharply demarcated loss of the RPE band on SD-OCT, an association with RPD, and choroidal thinning. The pathophysiology of AMD is multifactorial and among others age-related and AMD-associated alterations of Bruch’s membrane have been described including thickening and calcification, deposition of advanced glycation end-products, or lipid accumulation. It is therefore likely that in AMD, Bruch’s membrane pathology might at least partially contribute to the development and/or progression of GA. Other genetic and environmental factors known to be involved in the multifactorial AMD pathogenesis might also contribute to the variability of atrophic lesions in patients with PXE.

Based on large epidemiologic studies, the peak prevalence of GA without CNV in AMD is above 75 years of age with yearly progression rates around 1.5 to 2.1 mm² depending on different ethnic cohorts, inclusion criteria, and imaging modalities used for atrophy assessment. Atrophy in PXE occurs at younger age and overall shows faster progression rates (3.3 mm²/year), which might be explained by earlier onset and more severe pathology of Bruch’s membrane in

**TABLE. Progression of Atrophy in PXE**

<table>
<thead>
<tr>
<th>Number of Patients</th>
<th>Age, y</th>
<th>Atrophy Area at Baseline, mm²</th>
<th>Follow-Up, mo</th>
<th>Atrophy Area at Last Follow-Up, mm²</th>
<th>Atrophy Growth Rate, mm²/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CNV</td>
<td>7 (7)</td>
<td>52.7 ± 5.2 Range 47–62</td>
<td>5.5 ± 5.3 Range 0.8–13.1</td>
<td>38.7 ± 18.4 Range 14–70</td>
<td>15.8 ± 11.6 Range 2.7–35.3</td>
</tr>
<tr>
<td>Atrophy and CNV</td>
<td>13 (15)</td>
<td>52.6 ± 7.2 Range 37–64</td>
<td>4.5 ± 4.7 Range 0.5–17.9</td>
<td>36.4 ± 17.1 Range 18–70</td>
<td>9.1 ± 6.5 Range 0.8–19.1</td>
</tr>
<tr>
<td>Overall</td>
<td>20 (16)</td>
<td>54.2 ± 6.2 Range 37–64</td>
<td>4.9 ± 5.1 Range 0.5–17.9</td>
<td>37.2 ± 17.6 Range 14–70</td>
<td>12.0 ± 9.5 Range 0.8–35.3</td>
</tr>
</tbody>
</table>
patients with PXE compared to AMD. Due to the small sample size, we cannot comment on whether or not atrophy size at baseline would have an impact on the progression rate.

Further support for an important role of Bruch’s membrane in the development of atrophy is provided by two other monogenetic diseases with a primary pathology at the level of Bruch’s membrane: Sorsby fundus dystrophy and late-onset retinal degeneration (LORD). Both diseases are characterized by early onset atrophy of the outer retina and the RPE associated with choroidal thinning and RPD.3,4,39 (Cukras, et al. IOVS 2015;56:ARVO E-Abstract 3842) suggesting that these fundus features might represent a general response to pathology and/or dysfunction of the Bruch’s membrane. In the future, reliable measures of Bruch’s membrane pathology will be needed to further assess and quantify Bruch’s membrane changes in the aging eye, AMD, and other retinal diseases.

So far, there is only limited information on the impact of current or past CNV activity on growth of atrophic lesions. The commonly indistinct borders between the atrophy and CNV-associated changes impede reliable measurements of the lesion size. Moreover, the atrophy cannot spread toward areas where the RPE and the retina have already been damaged by a CNV, limiting measurable atrophy growth to areas away from the CNV or subretinal fibrosis. Also, because CNV commonly occurs at earlier disease stages than atrophy, Bruch’s membrane changes may be less extensive with lesser contribution to atrophy growth. The slower growth rate in patients with CNV has therefore to be interpreted with caution and would need validation from larger cohorts and/or other diseases such as AMD.

In patients without a confirmed diagnosis of PXE, other retinal diseases with similar phenotypic features need to be differentiated. In any case, the presence of peau d’orange (if visible) and peripheral comet lesions (if present), as well as more or less obvious characteristic dermal changes point toward the diagnosis of PXE.14 The same applies to angiod streaks that may be visible outside the area of atrophy and on OCT imaging within atrophic areas.40 In AMD, drusen precede the development of GA that usually has a later onset than in PXE. RPD occur in both diseases and may not be used to precede the development of GA that usually has a later onset. Bruch’s membrane sparing, 41 Sorsby fundus dystrophy and LORD.4 In the latter two, there are usually other family members affected due to an autosomal-dominant inheritance.42–45 Stargardt’s disease characteristically shows peripapillary sparing,44 whereas there is usually atrophy surrounding the disc in patients with PXE.

The results of this study reveal novel insights into the natural history of the ocular phenotype of PXE and support an important contribution of Bruch’s membrane pathology in the pathogenesis of atrophy of the outer retina and the RPE. This indicates Bruch’s membrane as a promising therapeutic target to prevent vision loss in patients with a variety of retinal diseases associated with atrophic lesions including GA in AMD.

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