Prevalence, Natural Course, and Prognostic Role of Refractile Drusen in Age-Related Macular Degeneration

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PURPOSE. To report prevalence, clinical characteristics, and prognostic significance of refractile drusen in eyes with intermediate age-related macular degeneration (AMD).

METHODS. Presence of refractile drusen by color fundus photography (CFP), corresponding findings by multimodal imaging, and longitudinal changes with annual examinations for up to 4 years were analyzed within a prospective natural history study of 98 eyes with non-late AMD of 98 patients (Age-Related Eye Disease Study [AREDS] stages 3 and 4).

RESULTS. A total of 115 refractile drusen were detected at baseline in 20 eyes (20.4%). Refractile drusen typically showed hyperreflectivity by infrared (80.9%) and blue (93.9%) reflectance imaging, appearing more distinct when compared to CFP. Laminar intense hyperreflectivity of Bruch’s membrane was detected in 31 lesions by spectral-domain optical coherence tomography and was strongly related to atrophy development (23 out of 31 lesions). Presence of refractile drusen at baseline was overall associated with later development of geographic atrophy (GA) (9/20 eyes versus 6/78 eyes, P < 0.001). Spontaneous regression without evident atrophy occurred in seven lesions.

CONCLUSIONS. Refractile drusen are a relative common phenotype in intermediate AMD and appear to confer risk for the development of late AMD. While not all lesions develop late AMD and regression may also occur, distinct subphenotypes as identified by multimodal imaging may not only be visible earlier but also be topographically associated with the risk for GA development. Recognizing the characteristic pattern on multimodal imaging would inform physicians for identification of the lesion and its clinical history.

Keywords: calcified drusen, crystalline drusen, precursor, intermediate age-related macular degeneration, geographic atrophy, biomarker

Age-related macular degeneration (AMD) is the leading cause of blindness in developed countries.1 Patients with untreated late AMD suffer loss of visual acuity, or central scotoma. Late AMD can manifest with choroidal neovascularization (CNV) and/or geographic atrophy (GA). While CNV can be treated with anti-vascular endothelial growth factor therapy, untreated late AMD suffer loss of visual acuity, or central scotoma. Late AMD can manifest with choroidal neovascularization (CNV) and/or geographic atrophy (GA). While CNV can be treated with anti-vascular endothelial growth factor therapy, there is yet no treatment available for GA due to AMD.2

When considering intervention for GA, prevention rather than treatment would be a desirable strategy as it is obviously challenging to restore cellular elements of the neurosensory retina once cell death has occurred. Considering that it may not be practical to treat a huge population with a potential risk of GA in a general AMD cohort, it appears prudent to identify prognostic biomarkers for the development of GA in order to deliver the intervention to appropriate subjects at higher risk.

Several morphologic features such as large soft drusen, confluent soft drusen, and pigmentary abnormalities are known biomarkers for the development of late AMD. The Age-Related Eye Disease Study (AREDS) has shown that antioxidant vitamins and minerals can decrease the risk for development of wet AMD in patients at high risk as defined by sterescopic color fundus photography (CFP), but the supplements had no significant effect in those with earlier stages.3 The result highlights the importance of appropriate selection of subjects for a specific intervention to the disease.

The presence of calcified drusen, also known as “ossified drusen”4 or “crystalline drusen,”5 represents a funduscopically visible sign that has been noted in both earlier and advanced stages of AMD.4 These “calcified drusen” are characterized by glistening,6 also described as chalky white,7 shiny,7 or calcific4 appearance by funduscopy or CFP. Polychromatic or golden sparkling appearance is presumed to be associated with cholesterol deposits,8 but distinction of the composition based on appearance is not easy. Thus, the term refractile drusen, without presumption concerning the material, has recently been proposed by Suzuki and coworkers.6

The presence of refractile material, which appeared during the regression of drusen, was associated with the development of GA during a mean time period of 2.5 years in a recent study by Klein and coworkers.9 Although the prevalence of refractile deposits in future GA area was only 25%,8 such lesions appeared to confer risk for the development of GA in a relatively short period. Another study investigating the natural history of drusenoid pigment epithelial detachment reported the increase in prevalence of refractile (calcified) drusen from 4% to 36% in 5 years, indicating that development of refractile...
In the present study, we defined refractile drusen (arrows) solely based on CFP. Refractile drusen is associated with the pathophysiologic processes leading to advanced AMD.9

Despite their potential clinical relevance, little is currently known about refractile drusen including the exact molecular composition and pathophysiology, natural history, topography, and prognostic relevance.

In the present study, we investigated the prevalence, clinical characteristics, and natural history of refractile drusen in a prospective longitudinal natural history study using state-of-the-art multimodal imaging. Particularly, we explored the association of refractile drusen and development of GA.

**Methods**

**Patient Selection**

The imaging database of the MODIAMD (Molecular Diagnostics of Age-related Macular Degeneration) study (www.modiamd.de; in the public domain) was screened for eyes with refractile drusen. The MODIAMD study is a prospective, noninterventional, observational, longitudinal natural history study in patients at high risk for developing late-stage AMD in the study eye.10,11 Subjects were recruited between November 2010 and September 2011 at the Department of Ophthalmology, University of Bonn, Germany. The investigation followed the tenets of the Declaration of Helsinki and was approved by the local ethics committee (Ethik-Kommission der Universität Bonn Lfd-Nr. 175/10). Informed consent was obtained from each patient after explanation of the nature and possible consequences of the study.

The detail of the study design was presented in previous reports.10,11 Briefly, inclusion criteria were age > 50 years and manifesting retinal changes classified as AREDS stage either 3 or 4, that is, having at least one eye without advanced AMD that would be considered to be at high risk for developing late stages of the disease. Exclusion criteria comprised any ocular disease that may confound the assessment of the retina other than AMD (e.g., diabetic retinopathy, glaucoma, retinal vessel occlusion, retinal dystrophies, and uveitis).

In patients with AREDS stage 3, the eye with more pronounced changes (larger drusen area and more pigmented changes) was selected as study eye. In patients with AREDS stage 4, the eye without advanced AMD was selected as the study eye.

Subjects underwent annual examinations including measurement of best-corrected visual acuity using Early Treatment Diabetic Retinopathy Study (ETDRS) charts and extensive retinal imaging. The study is still ongoing, and the present study included longitudinal data obtained over 4 years.

**Imaging Protocol**

Retinal imaging was performed according to standardized operating procedures as previously described in detail.10,11 It included acquisition of three-field CFP with a standard fundus camera (Visucam 500; Carl Zeiss Meditec AG, Jena, Germany) and multimodal combined confocal scanning laser ophthalmoscopy (cSLO) and spectral-domain optical coherence tomography (SD-OCT) imaging (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany). For the latter, central near-infrared (IR), blue reflectance (BR), and three-field blue-peak fundus autofluorescence (FAF) imaging as well as raster SD-OCT scanning was carried out. To improve the quality of images and reproducibility, Automatic Real Time (ART) technology and progression mode equipped with the device were routinely employed. The standard SD-OCT raster consisted of 19 B-scans (distance between neighboring B-scan approximately 240 μm, field size 20° × 15°). In order to adapt to further technical developments, a second and denser SD-OCT raster scan was included in the imaging protocol in June 2011. It was additionally performed at all visits (including baseline) from subject no. 055 onward and also in the previously enrolled subjects starting from the first follow-up visit at month 12. This latter SD-OCT raster consisted of 145 B-scans (distance approximately 30 μm).

**Analysis of the Images**

Color fundus photography at baseline and at follow-up visits was screened for the presence of refractile drusen by two independent graders (AO, MO). Refractile drusen were defined as drusen with deposition of yellowish-white glistening material beneath the retina. Presence or absence of refractile drusen was determined solely on CFP without taking other imaging modalities into account. Only lesions judged as refractile drusen by both graders were included (Fig. 1).

In all eyes with refractile drusen, combined cSLO+SD-OCT images from all visits were further investigated. Development of GA was defined as an area of complete loss of outer retinal layers by SD-OCT and a severely reduced FAF signal having minimum area of 0.05 mm². When the minimum distance from fovea to GA was less than 500 μm, the lesion was defined as central GA.

**Statistics**

Statistical analysis was carried out using IBM SPSS Statistics version 22 (IBM, Armonk, NY, USA). Results of statistical hypothesis tests were considered significant if $P$ values were smaller than 0.05. Patients with and without refractile drusen were compared at baseline using unpaired $t$-tests for normally distributed variables and the Mann-Whitney $U$ test for not normally distributed continuous variables. Categorical variables were analyzed with the exact Fisher test or the Trend test.
Refractile Drusen and GA Development

TABLE. Clinical Characteristics of Subjects With or Without Refractile Drusen

<table>
<thead>
<tr>
<th></th>
<th>Refractile Drusen Present, n = 20</th>
<th>No Refractile Drusen, n = 78</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>71.9 ± 7.0</td>
<td>73.4 ± 7.2</td>
<td>0.388</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
<td>0.108</td>
</tr>
<tr>
<td>Male</td>
<td>3 (15.0)</td>
<td>27 (34.6)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>17 (85.0)</td>
<td>51 (65.4)</td>
<td></td>
</tr>
<tr>
<td>ETDRS (letters)</td>
<td>78.5 (65.0; 85.0)</td>
<td>79.0 (56.0; 91.0)</td>
<td>0.642</td>
</tr>
<tr>
<td>AREDS category (%)</td>
<td></td>
<td></td>
<td>0.074</td>
</tr>
<tr>
<td>Category III</td>
<td>8 (40.0)</td>
<td>15 (19.2)</td>
<td></td>
</tr>
<tr>
<td>Category IV</td>
<td>12 (60.0)</td>
<td>63 (80.8)</td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>156.2 ± 9.2</td>
<td>167.4 ± 7.4</td>
<td>0.547</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>67.2 ± 12.8</td>
<td>73.6 ± 12.9</td>
<td>0.052</td>
</tr>
<tr>
<td>BMI</td>
<td>24.2 ± 3.5</td>
<td>26.2 ± 3.8</td>
<td>0.038</td>
</tr>
<tr>
<td>Smoking history (%)</td>
<td></td>
<td></td>
<td>0.602</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>12 (60.0)</td>
<td>49 (62.8)</td>
<td></td>
</tr>
<tr>
<td>Former smoker</td>
<td>5 (25.0)</td>
<td>22 (28.2)</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>3 (15.0)</td>
<td>7 (9.0)</td>
<td></td>
</tr>
</tbody>
</table>

Exact Fisher test was used for sex and AREDS category. Smoking history was analyzed with Cochran Armitage Trend Test. Mann-Whitney U test was used for ETDRS. The other items were compared with unpaired t-test.

RESULTS

Patient Characteristics

A total of 98 subjects (mean age 73.1 ± 7.1 years, 68 female) were initially enrolled in the MODIAMD study with a median visual acuity of 79.0 (56.0; 91.0) ETDRS letters at baseline. Twenty-three patients (23.5%) had AREDS stage 3 and 75 (76.5%) stage 4. At the year 4 annual study visit, 38 subjects were still in the study. Of the remaining 60 of 98 subjects included at baseline, 34 had developed late AMD (15 GA, 19 CNV), while 26 subjects had dropped out due to various reasons (e.g., lost to follow-up, consent withdrawal).

A total of 115 refractile drusen were identified in 20 patients (20.4%, 95% confidence interval [12.4; 28.4%] at baseline [median 3.5 per patient, range, 1–17]). Involvement of the fovea (central ETDRS subfield) was seen in nine (45.0%) subjects, while refractive drusen occurred only in the parafovea in the remaining 11 subjects (55.0%). The Table shows comparison between those with and without refractile drusen at baseline. Patients with refractile drusen showed significantly smaller body mass index (BMI). In addition, there was a tendency to be female, to be light in weight, and to have AREDS stage 4.

Characteristic Manifestation of Refractile Drusen Beyond Color Fundus Photography

Refractile drusen generally showed hyperreflective appearance in IR (95/115, 80.9%) and BR (108/115, 93.9%) images (Fig. 2). The intensity and the demarcation of refractile material were inconsistent as compared to CFP; that is, the detection of individual lesions was sometimes improved and in other cases not as clear. Corresponding FAF imaging showed mildly increased intensities, a mottled signal, or a rather decreased signal. However, looking at FAF images alone without any further information from other modalities or clinical examination, the presence of refractile material was not clearly detectable. The distinctive feature as seen by SD-OCT imaging was a laminar intense hyperreflectivity at the level of Bruch’s membrane similar in appearance to findings recently reported in the context of regressing drusen.12 Of the 94 refractile drusen that were located within the SD-OCT scan field, 31 (33.0%) lesions showed this characteristic SD-OCT appearance.

Development of Refractile Drusen

Refractile drusen developed de novo in six cases during the follow-up. Representative cases are shown in Figures 3 and 4. The refractile drusen were initially unrecognizable on fundus photography (Fig. 3). Moderate hyperreflectivity was occasionally noted in BR and IR images even at this stage (Fig. 3; Supplementary Fig. S1). The FAF signal at the site of lesions tended to be mildly increased. During the early stage, characteristic laminar hyperreflectivity at the level of Bruch’s membrane was not identified by SD-OCT, although hyperreflective appearance toward the inner retina at the top of drusen, previously named hyperreflective pyramidal structures, was sometimes observed (Fig. 3; Supplementary Fig. S1).13

As the refractile lesions become clearly visible on fundus photographs, hyperreflective appearance became more evident in IR and BR images. The characteristic laminar hyperreflective finding, if present, became detectable by SD-OCT. Of note, among the 31 lesions with laminar hyperreflective material at the level of Bruch’s membrane, 7 had already atrophy at baseline, 16 developed atrophy, 6 developed exudative changes during the follow-up, and 2 were lost to follow-up. Thus, at least 23/31 (74.2%) of such lesions were spatially confined to atrophy. These observations went along with progressive reduction of FAF intensities (Fig. 5).

Refractile deposits in a dot-like pattern developed in three cases after the collapse of drusenoid pigment epithelium detachment (Fig. 4). Such refractile deposits also showed hyperreflective appearance in IR and BR images. Hyperreflectivity at the level of Bruch’s membrane was not noted.

Regression of Refractile Drusen

Although development of atrophy was the typical consequence of the presence of refractile drusen as described above, seven regressed spontaneously without evident atrophy or exudative changes at least within the study period (Fig. 5). We noted temporal regression of refractile appearance in another 12 lesions; however, 3 became refractile again, 2 developed exudative changes, 1 developed GA later, and 6 were lost to follow-up.

Presence of Refractile Drusen and the Clinical Relevance

A total of nine eyes with refractile deposits and six eyes without refractile deposits at baseline developed GA over time, respectively (59.1% vs. 10.4%, P < 0.001) (Fig. 6). In addition, the development of GA was more frequent in cases with central refractile drusen at baseline (7/9 vs. 2/11 cases, P = 0.022). No statistically significant difference was found for CNV development (3 eyes versus 16 eyes, P = 0.801). Taken together, the presence of refractile drusen at baseline was associated with development of late AMD in general (either GA or CNV, Fig. 6).
DISCUSSION

In the present study, we report phenotypic characteristics and longitudinal morphologic changes of refractile drusen and their impact for the development of GA in eyes with non-late-stage AMD using multimodal imaging.

The results suggest that the presence of refractile drusen may represent a prognostic biomarker for conversion to late AMD, particularly development of central GA. This finding would be in accordance with the report by Klein and coworkers,8 who found a topographic association of refractile deposits and later development of GA in a substudy of the AREDS cohort, although this study did not directly compare eyes with and without refractile drusen to each other. Presence of refractile drusen, especially central refractile drusen, was associated with higher incidence of central GA in the current study. Meanwhile, development of GA in patients with noncentral refractile drusen (25.0%, based on survival analysis) was not as frequent as in those with central refractile drusen (77.8%) even though the incidence rate was higher than in those without refractile drusen (10.4%). The development of evident GA with complete loss of outer retinal tissue at the site of refractile drusen is different from retinal degeneration found in association with reticular drusen. While also representing a common AMD phenotype, reticular drusen have not been associated only with central GA,14–17 but also with development of outer retinal atrophy, that is, retinal thinning in between the inner plexiform layer and the retinal pigment epithelium.18 It may be speculated that refractile drusen, as compared to reticular drusen, would induce GA in a more area-specific manner and affect RPE more severely.

Refractile drusen generally showed multiple hyperreflective dots in IR and BR images. However, the degree of hyperreflectivity seemed not to be consistent among drusen. Sometimes definite refractile drusen on CFP showed only a slight hyperreflectivity, while in other cases cSLO hyperreflectivity was detectable before refractile drusen became visible by CFP. In addition, some drusen showed hyperreflective dots in both IR and BR images and other drusen showed them only in either modality. The discrepancy might be due to vertical location of the refractile material considering that blue light reflects more in superficial layers and infrared light reaches more in deeper layers. Alternatively, the difference might be due to the variety in reflective properties of accumulating material. The clinical significance of such different appearance should be investigated in future research.

In the current study, we could confirm the previous observation about the characteristic association of refractile drusen with laminar hyperreflectivity at the level of Bruch’s membrane (arrow). Scale bars indicate 200 μm.

The partly mild increased FAF signal is unlikely caused by autofluorescence properties, but rather might be the cause of pseudofluorescence phenomena; that is, the strong reflectivity of the reflective material would not have been blocked by the interference filter. While FAF imaging alone was not able to specifically detect the presence of refractile material, the development of decreased FAF intensities occurred with the development of signs of atrophy in other modalities.

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In the current study, we could confirm the previous observation about the characteristic association of refractile drusen with laminar hyperreflectivity at the level of Bruch’s membrane by SD-OCT.12 While earlier reports were based on cross-sectional data, we could track not only the appearance
but also the disappearance of this feature along with occurrence and regression of refractile material. Importantly, the strong topographic association with laminar hyporeflectivity at the level of Bruch’s membrane and later development of atrophy indicates that the former is a robust biomarker for development of late AMD.

Various age-related changes have been reported to occur in Bruch’s membrane including increased thickness, lipid accumulation, calcification, and iron as well as hydroxyapatite deposition. The SD-OCT finding might reflect the calcified components. Interestingly, an increase of reflectivity at the level of Bruch’s membrane was reported in pseudoxanthoma elasticum, in which disease calcification of Bruch’s membrane is a major hallmark. Considering that the deposition of such material would result in resistance to hydraulic conductivity, an important factor for viability of the RPE and the choroid, the development of GA in such lesions would be conceivable. The notion is in line with a previous study that confirmed calcification in macular drusen and stated that such change represents end-stage drusen. The longitudinal analysis of this study overall strongly suggests that refractile material represents a toxic byproduct of RPE–photoreceptor metabolism that may originate from a chronic defect in normal cell function.

Suzuki and associates proposed that there are concentric zones and that the appearance of refractile drusen varies depending on the zone. However, we could not identify such zone (i.e., appearance and development atrophy was not associated to concentric zones around the fovea in our cohort). The difference would be partly explained by the study
FIGURE 4. Development of refractile drusen in the presence of the collapse of a pigment epithelium detachment (PED; for general figure description, see Fig. 3). Drusenoid PED with hyperpigmentation was noted at baseline and year 1 (first and second columns). At year 2, collapse of drusenoid PED and development of atrophy were noted (third column). In addition, refractile deposits were observed on BR (blue arrow) and IR (yellow arrow) images as well as CFP (arrowhead). GA enlarged at year 3 without remarkable change of refractile deposits (square).
population; the average age of their study population was 82.9 years and is much older than that in the present study (73.5 years). Their observation might represent more advanced stages of the disease. Considering that the number of patients is limited both in the present study (20 eyes, 20 patients) and in their study (14 eyes of 10 patients), further studies are needed to clarify the entire course of the disease.

Various limitations of the study need to be considered. Firstly, the criteria for the presence of refractile drusen are based on fundus photography but not on quantitative criteria. Secondly, dropout and development of GA/CNV limited the observation of longitudinal changes. Since development of GA or CNV was the primary outcome of the MODIAMD study, patients who once developed either were followed only at physicians' discretion. Thirdly, six cases developed refractile drusen during the follow-up but were classified as no refractile drusen at baseline. Considering that some of these newly developed refractile drusen resulted in GA in the study period as shown in Figure 3, the present result might underestimate the effect of refractile drusen on GA development. Relatively small sample size is another limitation of the study, despite prospective long-term follow-up. In addition, the majority of cases (75/98, 76.5%) are AREDS 4, and that indicates some selection bias. Nevertheless, the 4-year incidence of GA was dramatically different depending on the presence of refractile drusen (59.1% vs. 10.4%, $P < 0.001$).

In summary, the present study described the detailed characteristics of refractile drusen and its natural course. The study also confirmed the association of refractile drusen and development of GA. Since the presence of refractile drusen is relatively easily confirmed by funduscopy or fundus photography, the present results would be relevant in clinical practice. In addition, the findings would be useful when recruiting particularly high-risk patients for intervention trials for GA.
Further studies are warranted for elucidating the etiology and composition of refractile drusen.

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